

FORM PTO-1390 (REV 11-98)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 127-01	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 10/018418	
INTERNATIONAL APPLICATION NO. PCT/AU00/00385		INTERNATIONAL FILING DATE 28 April 2000 (28.04.00)		PRIORITY DATE CLAIMED 29 April 1999 (29.04.99)	
TITLE OF INVENTION NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES THEREFOR GENES ENCODING WHEAT STARCH SYNTHASES AND USES THEREFOR (as amended)					
APPLICANT(S) FOR DO/EO/US Matthew MORELL, Zhongyi LI, Sadequr RAHMAN, Rudolph APPELS					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)): <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 					
Items 11. To 16. below concern document(s) or information included:					
<ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input checked="" type="checkbox"/> Other items or information: Sequence Listing on diskette (write protected) Sequence Listing as paper copy (pages 1--67) Statement under 37 CFR 1.821-1825 					
<div style="float: right; text-align: right;"> <p>I hereby certify that this correspondence is being deposited with the United States Postal Service as "Express Mail" in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on</p> <p><u>29 October 2001</u></p> <p>(Date of Deposit)</p> <p><u>B. Kroge</u></p> <p>Name of Applicant, Assignee or Registered Representative</p> <p><u>B. Kroge</u></p> <p>Signature</p> <p><u>29 October 2001</u></p> <p>Date of signature</p> <p><u>EL 527 990 190 US</u></p> <p>Express Mail Receipt No.</p> </div>					

(January 1999)

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) <div style="font-size: 1.5em; font-weight: bold;">10/018418</div>		INTERNATIONAL APPLICATION NO. PCT/AU00/00385		ATTORNEY'S DOCKET NUMBER 127-01	
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 1,040	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	66-20 =	46	X \$18.00	\$ 828	
Independent claims	5-3 =	2	X \$78.00	\$ 156	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+\$260.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 2,154	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$ N/A	
SUBTOTAL =				\$	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$ N/A	
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$ N/A	
TOTAL FEES ENCLOSED =				\$ 2,154	
				Amount to be: refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$2,154.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. 07-1969 in the amount of \$_____ to cover the above fees. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 07-1969 . A duplicate copy of this sheet is enclosed. NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status. SEND ALL CORRESPONDENCE TO: GREENLEE, WINNER and SULLIVAN, P.C. 5370 Manhattan Circle, Suite 201 Boulder, CO 80303 Phone: 303-499-8080 Fax: 303-499-8089					
				<div style="text-align: center;"> SIGNATURE </div> <div style="display: flex; justify-content: space-between;"> Name: Donna M. Ferber Registration No.: 33,878 </div>	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: :
Morrell et al. : Group Art Unit: Not yet assigned
Serial No: Not yet assigned : Examiner: Not yet assigned
Filed: October 29, 2001
For: GENES ENCODING WHEAT STARCH
SYNTHASES AND USES THEREFOR
(as amended)

CERTIFICATE OF MAILING	
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as EXPRESS MAIL in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231.	
29 October 2001	B. Kroge
Date	B. Kroge
Express Mail Receipt No: EL 827 990 190 US	

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Please enter the following amendments:

In the Title:

Rewrite the title as follows:

Genes Encoding Wheat Starch Synthases and Uses Therefor

In the Claims:

Rewrite claims 22, 26, 28, 32, 33, 41, 43, 44, 46, 47, 50, 52, 53, 54, 57 and 59 as follows:

22. (Once amended) A method comprising:

(i) hybridising single-stranded or double-stranded mRNA, cDNA or genomic DNA with a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence according to any one of claim 1;

- (b) a probe or primer derived from a nucleotide sequence according to subparagraph (a) and comprising at least about 15 contiguous nucleotides of said nucleotide sequence in length; and
 - (ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.
- 26. (Once amended) A method of assaying for the presence or absence of a wheat starch synthase polypeptide in a plant or a plant extract or isolated nucleic acid sample, said method at least comprising performing the method according to claim 22.
- 28. (Once amended) A method of marker-assisted breeding and/or selection of a plant at least comprising performing the method according to claim 22.
- 32. (Once amended) A plant produced by the method according to claim 28 wherein said plant expresses a wheat starch synthase polypeptide at a desired level detectable using said method.
- 33. (Once amended) A method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing in said plant a nucleic acid molecule for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified, wherein said nucleic acid molecule is selected from the group consisting of:
 - (i) the isolated nucleic acid molecule according to claim 1;
 - (ii) a fragment of (i) which comprises a nucleotide sequence capable of being expressed to down-regulate the expression of an endogenous wheat starch synthase isoenzyme of said plant; and
 - (iii) a fragment of (i) which encodes a functional wheat starch synthase isoenzyme of said plant.
- 41. (Once amended) A plant carrying the isolated nucleic acid molecule according to claim 1 as an exogenous complement to its genome
- 43. (Once amended) A propagule of the plant according to claim 41 wherein said propagule carries the introduced nucleic acid molecule present in said plant.

- 二、四、六、八、十、十二、十四、十六、十八、二十

Please add the following new claims 60 - 66:

60. (New) A modified starch derived from the plant according to claim 41 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.
61. (New) A food product comprising the modified starch according to claim 60.

Delete pages 1-59 of the existing Sequence Listing as filed in PCT/AU00/00385 and replace with new Sequence Listing pages 1-67.

The title has been amended to delete the initial word "Novel."

Claims 22, 26, 28, 32, 33, 41, 43, 44, 46, 47, 50, 52, 53, 54, 57 and 59 have amended to remove multiple dependency. New claims 60-66, which are supported by the original claims prior to the present amendment, have been entered. None of the amendments made herein constitutes the addition of new matter.

The Sequence Listing has been rewritten to U.S. Patent Office rules. All sequence information was present in the Sequence Listing which was part of PCT AU00/00385. Accordingly the present submission does not add new matter.

In view of the foregoing, it is submitted that this case is in condition for allowance, and passage to issuance is respectfully requested.

If there are any outstanding issues related to patentability, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

It is believed that this amendment does not necessitate the payment of any fees under 37 C.F.R. 1.16-1.17. If this is incorrect, however, please charge any fee due under the foregoing Rules to Deposit Account No. 07-1969.

Respectfully submitted,



Donna M. Ferber
Reg. No. 33,878

GREENLEE, WINNER AND SULLIVAN, P.C.
5370 Manhattan Circle, Suite 201
Boulder, CO 80303
Telephone (303) 499-8080
Facsimile: (303) 499-8089
Email: winner@greenwin.com

Attorney docket No.127-01
bnk: October 29, 2001

Marked up version of amended paragraph(s) and claim(s) in attached Amendment.

Docket No.: 127-01
 Filed: October 29, 2001

In the Title:

[Novel] Genes Encoding Wheat Starch Synthases and Uses Therefor

In the claims:

22. (Once amended) A method comprising:
 - (i) hybridising single-stranded or double-stranded mRNA, cDNA or genomic DNA with a nucleotide sequence selected from the group consisting of:
 - (a) the nucleotide sequence according to [any one of claims 1 to 9] claim 1;
 - (b) a probe or primer derived from a nucleotide sequence according to subparagraph (a) and comprising at least about 15 contiguous nucleotides of said nucleotide sequence in length; and
 - (ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.
26. (Once amended) A method of assaying for the presence or absence of a wheat starch synthase polypeptide in a plant or a plant extract or isolated nucleic acid sample, said method at least comprising performing the method according to [any one of claims 22 to 25.] claim 22.
28. (Once amended) A method of marker-assisted breeding and/or selection of a plant at least comprising performing the method according to [any one of claims 22 to 25.] claim 22.
32. (Once amended) A plant produced by the method according to [any one of claims 28 to 31] claim 28 wherein said plant expresses a wheat starch synthase polypeptide at a desired level detectable using said method.
33. (Once amended) A method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing in said plant a nucleic acid molecule for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified, wherein said nucleic acid molecule is selected from the group consisting of:
 - (i) the isolated nucleic acid molecule according to [any one of claims 1 to 9;] claim 1;
 - (ii) a fragment of (i) which comprises a nucleotide sequence capable of being expressed to down-regulate the expression of an endogenous wheat starch synthase isoenzyme of said plant; and
 - (iii) a fragment of (i) which encodes a functional wheat starch synthase isoenzyme of said plant.
41. (Once amended) A plant carrying the isolated nucleic acid molecule according to [ny one of claims 1 to 9] claim 1 as an exogenous complement to its genome

43. (Once amended) A propagule of the plant according to claim 41 [or 42] wherein said propagule carries the introduced nucleic acid molecule present in said plant.
44. (Once amended) A gene construct or vector which comprises the isolated nucleic acid molecule according to [any one of claims 1 to 9] claim 1 and one or more origins of replication
46. (Once amended) A gene construct or vector which comprises the probe or primer according to claim 10 [or 11] and one or more origins of replication.
47. (Once amended) A modified starch derived from the plant according to claim 32 [or 41] wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.
50. (Once amended) A food product comprising the modified starch according to [any one of claims 47 to 49.] claim 47.
52. (Once amended) The food product according to claim 50 [or 51] selected from the group consisting of: flour-based sauce; leavened bread; unleavened bread; pasta, noodle; cereal; snack food; cake; and pastry.
53. (Once amended) Use of the modified starch according to [any one of claims 47 to 49] claim 47 in the preparation of a food product for consumption by an animal or human.
54. (Once amended) A modified protein derived from the plant according to claim 32 [or 41] wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.
57. (Once amended) A non-food product comprising the modified protein according to [any one of claims 54 to 56.] claim 54.
59. (Once amended) Use of the modified protein according to [any one of claims 54 to 56] claim 54 in the preparation of a non-food product.

SEQUENCE LISTING

<110> Morell, Matthew
Li, Zhongyi
Rahman, Sadequr
Appels, Rudolph

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Asp Ala Gly Ile Asp Asp Ala Ala Pro Ala Arg Gln Pro Arg Ala Leu
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Tyr Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly Gly
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Leu Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly Leu
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Gly Trp Thr Phe Asp Arg Ala Glu Ala Gln Lys Leu Ile Glu Ala Leu
740 745 750

Gly His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg Gly
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Val	Ala	Glu	Arg	Arg	Asp	Pro	Val	Lys	Thr	Leu	Asp	Arg	Asp	Ala	Ala	
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Pro	Pro	Ser	Met	Asn	Gly	Thr	Pro	Val	Asn	Gly	Glu	Asn	Lys	Ser	Thr	
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Gly	Gly	Gly	Gly	Ala	Thr	Lys	Asp	Ser	Gly	Leu	Pro	Ala	Pro	Ala	Arg	
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Pro	Asp	Ser	Ala	Ala	Thr	Ile	Ser	Ile	Ser	Asp	Lys	Ala	Pro	Glu	Ser	
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Val	Ser	Ala	Ser	Ala	Pro	Arg	Leu	Asp	Ile	Asp	Ser	Asp	Val	Glu	Pro	
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Glu	Leu	Lys	Lys	Gly	Ala	Val	Ile	Val	Glu	Glu	Ala	Pro	Asn	Pro	Lys	
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Phe	Lys	Lys	Tyr	Ile	Gly	Phe	Glu	Glu	Pro	Val	Glu	Ala	Lys	Asp	Asp	
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Gly	Trp	Ala	Val	Ala	Asp	Asp	Ala	Gly	Ser	Phe	Glu	His	His	Gln	Asn	
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atg Met 345	gtt Val	gtg Val	gta Val	cca Pro	agg Arg	tat Tyr	ggg Gly	gac Asp	tat Tyr	gag Glu	gaa Glu	gcc Ala	tac Tyr	gat Asp	gtc Val 360	1168
gga Gly	gtc Val	cga Arg	aaa Lys	tac Tyr	tac Tyr	aag Lys	gct Ala	gct Ala	gga Gly	cag Gln	gat Asp	atg Met	gaa Glu	gtg Val	aat Asn	1216
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gaa Glu	att Ile	atg Met	aag Lys	cgc Arg	atg Met	att Ile	ttg Leu	ttc Phe	tgc Cys	aag Lys	gcc Ala	gct Ala	gtc Val	gag Glu	gtt Val	1360
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aaa Lys	gca Ala	tat Tyr	tac Tyr	agg Arg	gac Asp	cat His	ggg Gly	ttg Leu	atg Met	cag Gln	tac Tyr	act Thr	cgg Arg	tcc Ser	att Ile	1504
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ttc Phe	ccg Pro	ttc Phe	acc Thr	gag Glu	ttg Leu	cct Pro	gag Glu	cac His	tac Tyr	ctg Leu	gaa Glu	cac His	ttc Phe	aga Arg	ctg Leu	1600
tac Tyr 505	gac Asp	ccc Pro	gtg Val	ggg Gly	ggg Gly	gag Glu	cac His	gcc Ala	aac Asn	tac Tyr	ttc Phe	gcc Ala	gcc Ala	ggc Gly	ctg Leu 520	1648

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Leu Lys Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Arg Gln	
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Asn Asp Trp Lys Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu	
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Phe Ser Leu Gly Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala	
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Leu Gln Arg Glu Leu Gly Leu Gln Val Arg Ala Asp Val Pro Leu Leu	
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Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile Ala	
620 625 630	
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Asp Ala Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val Met Leu	
635 640 645	
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Gly Thr Gly Arg His Asp Leu Glu Ser Met Leu Arg His Phe Glu Arg	
650 655 660	
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Glu His His Asp Lys Val Arg Gly Trp Val Gly Phe Ser Val Arg Leu	
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Ala His Arg Ile Thr Ala Gly Ala Asp Ala Leu Leu Met Pro Ser Arg	
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Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr	
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Val Pro Val Val His Ala Val Gly Gly Val Arg Asp Thr Val Pro Pro	
715 720 725	
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Phe Asp Pro Phe Asn His Ser Gly Leu Gly Trp Thr Phe Asp Arg Ala	
730 735 740	

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 Glu Ala His Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr
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 Gln Asp Phe Ser Trp Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu
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 Lys Thr Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ala Pro Pro
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Ser	Gly	Leu	Pro	Ala	Pro	Ala	Arg	Ala	Pro	His	Pro	Ser	Thr	Gln	Asn	145	150	155
Arg	Val	Pro	Val	Asn	Gly	Glu	Asn	Lys	Ala	Asn	Val	Ala	Ser	Pro	Pro	165	170	175
Thr	Ser	Ile	Ala	Glu	Val	Val	Ala	Pro	Asp	Ser	Ala	Ala	Thr	Ile	Ser	180	185	190
Ile	Ser	Asp	Lys	Ala	Pro	Glu	Ser	Val	Val	Pro	Ala	Glu	Lys	Pro	Pro	195	200	205
Pro	Ser	Ser	Gly	Ser	Asn	Phe	Val	Val	Ser	Ala	Ser	Ala	Pro	Arg	Leu	210	215	220
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Ala	Val	Gln	Glu	Asp	Leu	Trp	Asp	Phe	Lys	Lys	Tyr	Ile	Gly	Phe	Glu	260	265	270
Glu	Pro	Val	Glu	Ala	Lys	Asp	Asp	Gly	Trp	Ala	Val	Ala	Asp	Asp	Ala	275	280	285
Gly	Ser	Phe	Glu	His	His	Gln	Asn	His	Asp	Ser	Gly	Pro	Leu	Ala	Gly	290	295	300
Glu	Asn	Val	Met	Asn	Val	Val	Val	Val	Ala	Ala	Glu	Cys	Ser	Pro	Trp	305	310	315
Cys	Lys	Thr	Gly	Gly	Leu	Gly	Asp	Val	Ala	Gly	Ala	Leu	Pro	Lys	Ala	325	330	335
Leu	Ala	Lys	Arg	Gly	His	Arg	Val	Met	Val	Val	Val	Pro	Arg	Tyr	Gly	340	345	350
Asp	Tyr	Glu	Glu	Ala	Tyr	Asp	Val	Gly	Val	Arg	Lys	Tyr	Tyr	Lys	Ala	355	360	365
Ala	Gly	Gln	Asp	Met	Glu	Val	Asn	Tyr	Phe	His	Ala	Tyr	Ile	Asp	Gly	370	375	380
Val	Asp	Phe	Val	Phe	Ile	Asp	Ala	Pro	Leu	Phe	Arg	His	Arg	Gln	Glu	385	390	395

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Asp	Ile	Tyr	Gly	Gly	Ser	Arg	Gln	Glu	Ile	Met	Lys	Arg	Met	Ile	Leu				
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Phe	Cys	Lys	Ala	Ala	Val	Glu	Val	Pro	Trp	His	Val	Pro	Cys	Gly	Gly				
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Val	Pro	Tyr	Gly	Asp	Gly	Asn	Leu	Val	Phe	Ile	Ala	Asn	Asp	Trp	His				
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Thr	Ala	Leu	Leu	Pro	Val	Tyr	Leu	Lys	Ala	Tyr	Tyr	Arg	Asp	His	Gly				
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Leu	Met	Gln	Tyr	Thr	Arg	Ser	Ile	Met	Val	Ile	His	Asn	Ile	Ala	His				
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Gln	Gly	Arg	Gly	Pro	Val	Asp	Glu	Phe	Pro	Phe	Thr	Glu	Leu	Pro	Glu				
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His	Tyr	Leu	Glu	His	Phe	Arg	Leu	Tyr	Asp	Pro	Val	Gly	Gly	Glu	His				
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Ala	Asn	Tyr	Phe	Ala	Ala	Gly	Leu	Lys	Met	Ala	Asp	Gln	Val	Val	Val				
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Gln	Asp	Val	Gln	Leu	Val	Met	Leu	Gly	Thr	Gly	Arg	His	Asp	Leu	Glu				
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Ser	Met	Leu	Arg	His	Phe	Glu	Arg	Glu	His	His	Asp	Lys	Val	Arg	Gly				
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Trp	Val	Gly	Phe	Ser	Val	Arg	Leu	Ala	His	Arg	Ile	Thr	Ala	Gly	Ala				
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Asp Ala Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln
690 695 700

Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly
705 710 715 720

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725 730 735

Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala His Lys Leu Ile Glu Ala
740 745 750

Leu Gly His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg
755 760 765

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770 775 780

Ala Lys Leu Tyr Glu Asp Val Leu Leu Lys Ala Lys Tyr Gln Trp
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Ala	Ser	Ala	Pro	Gly	Ser	Asp	Thr	Val	Ser	Asp	Val	Glu	Gln	Glu	Leu	
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Lys	Lys	Gly	Ala	Val	Val	Val	Glu	Glu	Ala	Pro	Lys	Pro	Lys	Ala	Leu	
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Ser	Pro	Pro	Ala	Ala	Pro	Ala	Val	Gln	Glu	Asp	Leu	Trp	Asp	Phe	Lys	
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Lys	Tyr	Ile	Gly	Phe	Glu	Glu	Pro	Val	Glu	Ala	Lys	Asp	Asp	Gly	Arg	
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gct	gtc	gca	gat	gat	gcg	ggc	tcc	ttt	gaa	cac	cac	cag	aat	cac	gac	288
Ala	Val	Ala	Asp	Asp	Ala	Gly	Ser	Phe	Glu	His	His	Gln	Asn	His	Asp	
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gct	gag	tgt	tct	ccc	tgg	tgc	aaa	aca	ggt	ggt	ctg	gga	gat	gtt	gcg	384
Ala	Glu	Cys	Ser	Pro	Trp	Cys	Lys	Thr	Gly	Gly	Leu	Gly	Asp	Val	Ala	
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Gly	Ala	Leu	Pro	Lys	Ala	Leu	Ala	Lys	Arg	Gly	His	Arg	Val	Met	Val	
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Val	Val	Pro	Arg	Tyr	Gly	Asp	Tyr	Glu	Glu	Pro	Thr	Asp	Val	Gly	Val	
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cga	aaa	tac	tac	aag	gct	gct	gga	cag	gat	atg	gaa	gtg	aat	tat	ttc	528
Arg	Lys	Tyr	Tyr	Lys	Ala	Ala	Gly	Gln	Asp	Met	Glu	Val	Asn	Tyr	Phe	
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cat	gct	tat	atc	gat	gga	gtt	gat	ttt	gtg	ttc	att	gac	gct	cct	ctc	576
His	Ala	Tyr	Ile	Asp	Gly	Val	Asp	Phe	Val	Phe	Ile	Asp	Ala	Pro	Leu	
			180					185					190			
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Phe	Arg	His	Arg	Glu	Glu	Asp	Ile	Tyr	Gly	Gly	Ser	Arg	Gln	Glu	Ile	
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Met	Lys	Arg	Met	Ile	Leu	Phe	Cys	Lys	Ala	Ala	Val	Glu	Val	Pro	Trp	
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Ile	Ala	Asn	Asp	Trp	His	Thr	Ala	Leu	Leu	Pro	Val	Tyr	Leu	Lys	Ala	
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Tyr	Tyr	Arg	Asp	His	Gly	Leu	Met	Gln	Tyr	Thr	Arg	Ser	Ile	Met	Val	
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Phe	Thr	Glu	Leu	Pro	Glu	His	Tyr	Leu	Glu	His	Phe	Arg	Leu	Tyr	Asp	
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gcg	gac	cag	gtt	gtc	gtg	gtg	agc	ccc	ggg	tac	ctg	tgg	gag	ctg	aag	1008
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Trp	Lys	Thr	Arg	Gly	Ile	Val	Asn	Gly	Ile	Asp	Asn	Met	Glu	Trp	Asn	
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Pro	Glu	Val	Asp	Ala	His	Leu	Lys	Ser	Asp	Gly	Tyr	Thr	Asn	Phe	Ser	
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Arg	Ile	Thr	Ala	Gly	Ala	Asp	Ala	Leu	Leu	Met	Pro	Ser	Arg	Phe	Val	
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Pro	Cys	Gly	Leu	Asn	Gln	Leu	Tyr	Ala	Met	Ala	Tyr	Gly	Thr	Val	Pro	
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Val	Val	His	Ala	Val	Gly	Gly	Leu	Arg	Asp	Thr	Val	Pro	Pro	Phe	Asp	
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ccc	ttc	aac	cac	tcc	ggg	ctc	ggg	tgg	acg	ttc	gac	cgc	gcc	gag	gcg	1632
Pro	Phe	Asn	His	Ser	Gly	Leu	Gly	Trp	Thr	Phe	Asp	Arg	Ala	Glu	Ala	
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ALCOA and EQUAL OPPORTUNITY

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Phe Lys Glu Ser Trp Arg Ala Leu Gln Glu Arg Gly Met Ser Gln Asp
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ttc agc tgg gag cac gcc gcc aag ctc tac gag gac gtc ctc gtc aag 1776
Phe Ser Trp Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu Val Lys
580 585 590

gcc aag tac cag tgg tgaacgctag ctgctagccg ctccagcccc gcatgcgtgc 1831
Ala Lys Tyr Gln Trp
595

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Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp Phe Lys
50 55 60

Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Arg
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Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp
85 90 95

Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala
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Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala
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Gly	Ala	Leu	Pro	Lys	Ala	Leu	Ala	Lys	Arg	Gly	His	Arg	Val	Met	Val	130	135	140
Val	Val	Pro	Arg	Tyr	Gly	Asp	Tyr	Glu	Glu	Pro	Thr	Asp	Val	Gly	Val	145	150	155
Arg	Lys	Tyr	Tyr	Lys	Ala	Ala	Gly	Gln	Asp	Met	Glu	Val	Asn	Tyr	Phe	165	170	175
His	Ala	Tyr	Ile	Asp	Gly	Val	Asp	Phe	Val	Phe	Ile	Asp	Ala	Pro	Leu	180	185	190
Phe	Arg	His	Arg	Glu	Glu	Asp	Ile	Tyr	Gly	Gly	Ser	Arg	Gln	Glu	Ile	195	200	205
Met	Lys	Arg	Met	Ile	Leu	Phe	Cys	Lys	Ala	Ala	Val	Glu	Val	Pro	Trp	210	215	220
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gtg Val	tat Tyr	tca Ser 155	ttg Leu	agc Ser	agt Ser	gta Val	atg Met 160	aag Lys	aag Lys	gaa Glu	gtg Val	gat Asp 165	gca Ala	gcg Ala	gac Asp	532
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caa Gln	gag Glu	aca Thr	ttg Leu	aga Arg 205	agt Ser	gtg Val	ata Ile	gta Val	gat Asp 210	gtg Val	atg Met	gat Asp	cat His	aat Asn 215	ggg Gly	676
act Thr	gta Val	caa Gln	gag Glu 220	aca Thr	ttg Leu	aga Arg	agt Ser	gtg Val 225	ata Ile	gta Val	gat Asp	gtg Val	atg Met	gat Asp 230	gat Asp	724
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Asp Asp Leu Pro Gly Gln Asn Gln Ser Ile Ile Gly Ser Tyr Lys Gln	
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Val Asp Asn Val Leu Arg Lys His Gln Ala Asp Arg Thr Gln Ala Val	
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Glu Leu Ser Ile Thr Glu Ile Gly Met Gly Arg Gly Asp Lys Ile Gln	
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Leu Ile Glu Asp Asp Gly Gln Tyr Glu Val Asp Glu Thr Ser Val Ser	
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Ala Glu Arg Arg Thr Gln Thr Glu Glu Gln Arg Arg Arg Lys Glu Ala	
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Asn	Gly	Lys	Ser	Glu	Gly	Trp	Phe	Arg	Cys	Ser	Phe	Asn	Leu	Trp	Met	
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His	Ser	Ser	Gly	Ala	Leu	Pro	Pro	Gln	Lys	Met	Val	Lys	Ser	Gly	Asp	
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ggg	ccg	ctc	tta	aaa	gca											

ALL INFORMATION CONTAINED HEREIN IS UNCLASSIFIED

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Asn Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile	
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Pro Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala	
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Lys Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro	
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Leu Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ser Asp	
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ttc ata att gtt cct tca atc ttc gaa ccc tgt ggc tta aca caa ctt	4612
Phe Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu	
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gtt gcc atg cgt tat gga tcg atc cct ata gtt cgg aaa act gga gga	4660
Val Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly	
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ctt cac gac aca gtc ttc gac gta gac aat gat aag gac cgg gct cgg	4708
Leu His Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg	
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tct ctt ggt ctt gaa cca aat ggg ttc agt ttc gac gga gcc gac agc	4756
Ser Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser	
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aat ggc gtg gat tat gcc ctc aac aga gca atc ggc gct tgg ttc gat	4804
Asn Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp	
1580 1585 1590	


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gcc cgt gat tgg ttc cac tcc ctg tgt aag agg gtc atg gag caa gac 4852
Ala Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp
      1595                1600                1605
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tgg tcg tgg aac cgg ccc gca ctg gac tac att gaa ttg tac cat gcc      4900
Trp Ser Trp Asn Arg Pro Ala Leu Asp Tyr Ile Glu Leu Tyr His Ala
      1610                      1615                      1620

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gct cga aaa ttc tgacacccaa ctgaaccaat gacaagaaca agcgcattgt 4952
Ala Arg Lys Phe
1625

gggatcgact agtcatacag ggctgtgcag atcgtcttgc ttcagttagt gccctcttca 5012

gtagttcca agcgactac agtcgtacat agctgaggat cctcttgctt cctaccaggg 5072

ggaacaaagc agaaatgcat gagtgcattg ggaagacttt tatgtatatt gttaaaaaaaaa 5132

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ccgcagtgac attctgtgag tagctttgta tattctctca tcttgtgaaa actaatgttc 5252

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gatatttaca tttgtggaaa aaaaaaaaaa aaaa 5346

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<211> 1628

<212> PRT

<213> Triticum aestivum

<400> 8

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Pro Phe Leu Met Asn Gly Arg Phe Thr Arg Ser Arg Thr Leu Arg Cys
35 40 45

Met Val Ala Ser Ser Asp Pro Pro Asn Arg Lys Ser Arg Arg Met Val
50 55 60

Pro	Pro	Gln	Val	Lys	Val	Ile	Ser	Ser	Arg	Gly	Tyr	Thr	Thr	Arg	Leu
65					70					75					80

Ile Val Glu Pro Ser Asn Glu Asn Thr Glu His Asn Asn Arg Asp Glu
85 90 95

Glu Thr Leu Asp Thr Tyr Asn Ala Leu Leu Ser Thr Glu Thr Ala Glu
100 105 110

Trp Thr Asp Asn Arg Glu Ala Glu Thr Ala Lys Ala Asp Ser Ser Gln
115 120 125

Asn	Ala	Leu	Ser	Ser	Ser	Ile	Ile	Gly	Glu	Val	Asp	Val	Ala	Asp	Glu
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Asp	Ile	Leu	Ala	Ala	Asp	Leu	Thr	Val	Tyr	Ser	Leu	Ser	Ser	Val	Met
145					150					155					160
Lys	Lys	Glu	Val	Asp	Ala	Ala	Asp	Lys	Ala	Arg	Val	Lys	Glu	Asp	Ala
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Phe	Glu	Leu	Asp	Leu	Pro	Ala	Thr	Thr	Leu	Arg	Ser	Val	Ile	Val	Asp
			180					185					190		
Val	Met	Asp	His	Asn	Gly	Thr	Val	Gln	Glu	Thr	Leu	Arg	Ser	Val	Ile
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Val	Asp	Val	Met	Asp	His	Asn	Gly	Thr	Val	Gln	Glu	Thr	Leu	Arg	Ser
210					215					220					
Val	Ile	Val	Asp	Val	Met	Asp	Asp	Ala	Ala	Asp	Lys	Ala	Arg	Val	Glu
225					230					235					240
Glu	Asp	Val	Phe	Glu	Leu	Asp	Leu	Ser	Gly	Asn	Ile	Ser	Ser	Ser	Ala
			245					250					255		
Thr	Thr	Val	Glu	Leu	Asp	Ala	Val	Asp	Glu	Val	Gly	Pro	Val	Gln	Asp
			260					265					270		
Lys	Phe	Glu	Ala	Thr	Ser	Ser	Gly	Asn	Val	Ser	Asn	Ser	Ala	Thr	Val
275							280					285			
Arg	Glu	Val	Asp	Ala	Ser	Asp	Glu	Ala	Gly	Asn	Asp	Gln	Gly	Ile	Phe
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Arg	Ala	Asp	Leu	Ser	Gly	Asn	Val	Phe	Ser	Ser	Ser	Thr	Thr	Val	Glu
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Val	Gly	Ala	Val	Asp	Glu	Ala	Gly	Ser	Ile	Lys	Asp	Arg	Phe	Glu	Thr
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Asp	Ser	Ser	Gly	Asn	Val	Ser	Thr	Ser	Ala	Pro	Met	Trp	Asp	Ala	Ile
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Asp	Glu	Thr	Val	Ala	Asp	Gln	Asp	Thr	Phe	Glu	Ala	Asp	Leu	Ser	Gly
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370					375					380					
Glu	Thr	Arg	Ser	Glu	Glu	Glu	Thr	Phe	Ala	Met	Asp	Leu	Phe	Ala	Ser
385					390					395					400
Glu	Ser	Gly	His	Glu	Lys	His	Met	Ala	Val	Asp	Tyr	Val	Gly	Glu	Ala
			405					410					415		

Asn Ala Arg Asn Tyr Asp Asn Asn Ala Arg Gln Asp Phe His Ala Ile
995 1000 1005

Leu Pro Asn Asn Asn Val Thr Glu Glu Gly Phe Trp Ala Gln Glu Glu
1010 1015 1020

Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu Arg Arg Glu Lys Glu Glu
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Thr Met Lys Arg Lys Ala Glu Arg Ser Ala Asn Ile Lys Ala Glu Met
1045 1050 1055

Lys Ala Lys Thr Met Arg Arg Phe Leu Leu Ser Gln Lys His Ile Val
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Tyr Thr Glu Pro Leu Glu Ile Arg Ala Gly Thr Thr Val Asp Val Leu
1075 1080 1085

Tyr Asn Pro Ser Asn Thr Val Leu Asn Gly Lys Ser Glu Gly Trp Phe
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Arg Cys Ser Phe Asn Leu Trp Met His Ser Ser Gly Ala Leu Pro Pro
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Gln Lys Met Val Lys Ser Gly Asp Gly Pro Leu Leu Lys Ala Thr Val
1125 1130 1135

Asp Val Pro Pro Asp Ala Tyr Met Met Asp Phe Val Phe Ser Glu Trp
1140 1145 1150

Glu Glu Asp Gly Ile Tyr Asp Asn Arg Asn Gly Met Asp Tyr His Ile
1155 1160 1165

Pro Val Ser Asp Ser Ile Glu Thr Glu Asn Tyr Met Arg Ile Ile His
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Ile Ala Val Glu Met Ala Pro Val Ala Lys Val Gly Gly Leu Gly Asp
1185 1190 1195 1200

Val Val Thr Ser Leu Ser Arg Ala Ile Gln Asp Leu Gly His Thr Val
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Glu Val Ile Leu Pro Lys Tyr Asp Cys Leu Asn Gln Ser Ser Val Lys
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Asp Leu His Leu Tyr Gln Ser Phe Ser Trp Gly Gly Thr Glu Ile Lys
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Val Trp Val Gly Arg Val Glu Asp Leu Thr Val Tyr Phe Leu Glu Pro
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Gln Asn Gly Met Phe Gly Val Gly Cys Val Tyr Gly Arg Asn Asp Asp
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Arg Arg Phe Gly Phe Phe Cys His Ser Ala Leu Glu Phe Ile Leu Gln
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Asn Glu Phe Ser Pro His Ile Ile His Cys His Asp Trp Ser Ser Ala
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Pro Val Ala Trp Leu Tyr Lys Glu His Tyr Ser Gln Ser Arg Met Ala
1315 1320 1325

Ser Thr Arg Val Val Phe Thr Ile His Asn Leu Glu Phe Gly Ala His
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Tyr Ile Gly Lys Ala Met Thr Tyr Cys Asp Lys Ala Thr Thr Val Ser
1345 1350 1355 1360

Pro Thr Tyr Ser Arg Asp Val Ala Gly His Gly Ala Ile Ala Pro His
1365 1370 1375

Arg Glu Lys Phe Tyr Gly Ile Leu Asn Gly Ile Asp Pro Asp Ile Trp
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Asp Pro Tyr Thr Asp Asn Phe Ile Pro Val Pro Tyr Thr Cys Glu Asn
1395 1400 1405

Val Val Glu Gly Lys Arg Ala Ala Lys Arg Ala Leu Gln Gln Lys Phe
1410 1415 1420

Gly Leu Gln Gln Thr Asp Val Pro Ile Val Gly Ile Ile Thr Arg Leu
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Thr Ala Gln Lys Gly Ile His Leu Ile Lys His Ala Ile His Arg Thr
1445 1450 1455

Leu Glu Ser Asn Gly His Val Val Leu Leu Gly Ser Ala Pro Asp His
1460 1465 1470

Arg Ile Gln Gly Asp Phe Cys Arg Leu Ala Asp Ala Leu His Gly Val
1475 1480 1485

Tyr His Gly Arg Val Lys Leu Val Leu Thr Tyr Asp Glu Pro Leu Ser
1490 1495 1500

His Leu Ile Tyr Ala Gly Ser Asp Phe Ile Ile Val Pro Ser Ile Phe
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Glu Pro Cys Gly Leu Thr Gln Leu Val Ala Met Arg Tyr Gly Ser Ile
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Pro Ile Val Arg Lys Thr Gly Gly Leu His Asp Thr Val Phe Asp Val
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Asp Asn Asp Lys Asp Arg Ala Arg Ser Leu Gly Leu Glu Pro Asn Gly
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Phe Ser Phe Asp Gly Ala Asp Ser Asn Gly Val Asp Tyr Ala Leu Asn
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Arg Ala Ile Gly Ala Trp Phe Asp Ala Arg Asp Trp Phe His Ser Leu
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<211> 3621
<212> DNA
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<222> (1) .. (3621)
<223> n can be a or g or c or t, and the encoded amino
acid cannot be assigned with certainty.
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<220>  
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<210> 10
 <211> 1059
 <212> PRT
 <213> Triticum aestivum

<220>
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 <223> Xaa is an amino acid which could not be identified
 with certainty.

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Gln	Lys	Arg	Ala	Ala	Glu	Gly	Gln	Met	Val	Val	Asn	Glu	Asp	Glu	Leu	
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Val	Glu	Gln	Asp	Ile	Gln	Gly	Ser	Pro	Gln	Asp	Val	Val	Asp	Pro	Gln	
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Ala	Leu	Lys	Val	Met	Leu	Gln	Glu	Leu	Ala	Glu	Lys	Asn	Tyr	Ser	Met	
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Ile	Asp	Leu	Tyr	Leu	Asn	Arg	Asp	Leu	Thr	Ala	Leu	Ala	Asn	Glu	Pro	
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Asp	Val	Val	Ile	Lys	Gly	Ala	Phe	Asn	Gly	Trp	Lys	Trp	Arg	Leu	Phe	
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Lys	Leu	Tyr	Ile	Pro	Lys	Glu	Ala	Tyr	Arg	Leu	Asp	Phe	Val	Phe	Phe	
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Lys	Glu	Lys	Gln	Arg	Glu	Leu	Glu	Lys	Leu	Ala	Met	Glu	Glu	Ala	Glu	
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Lys Lys Lys Leu Gln Ser Met Leu Ser Leu Ala Arg Thr Cys Val Asp
325 330 335

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340 345 350

Arg Leu Tyr Tyr Asn Arg Asn Ser Arg Pro Leu Ala His Ser Thr Glu
355 360 365

Ile Trp Met His Gly Gly Tyr Asn Asn Trp Ser Asp Gly Leu Ser Ile
370 375 380

Val Glu Ser Phe Val Lys Cys Asn Asp Lys Asp Gly Asp Trp Trp Tyr
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Ala Asp Gly Pro Ala Gly Asn Ala Arg Asn Tyr Asp Asn Asn Ala Arg
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Gln Asp Phe His Ala Ile Leu Pro Asn Asn Asn Val Thr Glu Glu Gly
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Phe Trp Ala Gln Glu Glu Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu
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Ser Gln Lys His Ile Val Tyr Thr Arg Thr Xaa Leu Lys Tyr Val Pro
500 505 510

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Ser Ser Gly Ala Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp Gly
545 550 555 560

Pro Leu Leu Lys Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met Met
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Asp Phe Val Phe Ser Glu Trp Glu Glu Asp Gly Ile Tyr Asp Asn Arg
580 585 590

Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly Gln Val Val Leu
885 890 895

Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg Leu
900 905 910

Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val Leu
915 920 925

Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ser Asp Phe
930 935 940

Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu Val
945 950 955 960

Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly Leu
965 970 975

Tyr Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg Ser
980 985 990

Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser Asn
995 1000 1005

Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp Ala
1010 1015 1020

Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp Trp
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Ser Trp Asn Arg Pro Ala Leu Asp Tyr Ile Glu Leu Tyr His Ala Ala
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Arg Lys Phe

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 <212> DNA
 <213> Triticum sp.

<400> 13

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<212> DNA
<213> Triticum sp.
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<210> 15
 <211> 871
 <212> DNA
 <213> Triticum sp.

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<210> 16
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 <213> Triticum sp.

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<210> 17

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:peptide motif

<400> 17

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<210> 18

<211> 10

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence:peptide motif

<400> 18

Ala Ala Gly Lys Lys Asp Ala Gly Ile Asp

1 5 10

<210> 19

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:peptide motif

<400> 19

Ala Thr Gly Lys Lys Asp Ala Gly Ile Asp

1 5 10

<210> 20

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:peptide motif

<400> 20

Ala Leu Gly Lys Lys Asp Ala Gly Ile Asp

1 5 10

<210> 21
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:peptide motif

<400> 21
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<210> 22
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:peptide motif

<400> 22
 Ala Leu Gly Lys Lys Asp Ala Leu
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<210> 23
 <211> 14
 <212> PRT
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<220>
 <223> Description of Artificial Sequence:peptide motif

<400> 23
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 1 5 10

<210> 24
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:peptide motif

<400> 24
 Ala Leu Gly Lys Lys Asp Ala Gly Ile Val Asp Gly Ala
 1 5 10

<210> 25
 <211> 23

<212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:oligonucleotide
 useful as a primer

<400> 25
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<210> 26
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
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 useful as a primer

<400> 26
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<210> 27
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 <213> Artificial Sequence

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 useful as a primer

<400> 27
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<210> 28
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 <212> DNA
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 useful as a primer

<400> 28
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<210> 29
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 useful as a primer

<400> 29
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<210> 30
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 <212> DNA
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<220>
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 useful as a primer

<400> 30
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<210> 31
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 useful as a primer

<400> 31
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<210> 32
 <211> 21
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 useful as a primer

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<210> 33
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 useful as a primer

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<210> 34
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useful as a primer

<400> 34
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<210> 35
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:peptide motif

<400> 35
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<210> 36
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:peptide motif

<400> 36
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Gln Asp Leu Gly His Asn Val Glu Val
20 25

<210> 37
<211> 9024
<212> DNA
<213> Triticum sp.

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10015418-050902
Rec'd PCT/PTO 07 MAY 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:

Morell et al.

: Group Art Unit: Not yet assigned

Serial No: 10/018,418

: Examiner: Not yet assigned

371 Filing date: October 29, 2001 : Confirmation No. 1169

Int. Filing Date: April 28, 2000

For: GENES ENCODING WHEAT STARCH
SYNTHASES AND USES THEREFOR
(as amended)

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as EXPRESS MAIL in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231.

7 May 2002

Date

B. Kroge

B. Kroge

Express Mail Receipt No: EL 818 653 764 US

SECOND PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Please enter the following amendments:

In the Specification:

At page 1, after the title, please insert:

CROSS REFERENCE TO RELATED APPLICATIONS

This application was filed under 35 U.S.C. 371, based on PCT/AU00/00385, which application was filed April 28, 2000 and claims priority from Australian Patent Application No. PQ0052/99 filed April 29, 1999.

REMARKS

Applicants have amended the application to include a paragraph providing cross reference to related applications

It is believed that this amendment does not necessitate the payment of any fees under 37 C.F.R. 1.16-1.17. If this is incorrect, however, please charge any fee due under the foregoing Rules to Deposit Account No. 07-1969.

Respectfully submitted,



Donna M. Ferber
Reg. No. 33,878

GREENLEE, WINNER AND SULLIVAN, P.C.
5370 Manhattan Circle, Suite 201
Boulder, CO 80303
Telephone (303) 499-8080
Facsimile: (303) 499-8089
Email: winner@greenwin.com

Attorney docket No. 127-01
bmk: May 7, 2002



10038418-050902
Rec'd PCT/PTO 09 MAY 2002

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of:

Morell et al. : Group Art Unit: Not yet assigned

Serial No: 10/018,418 : Examiner: Not yet assigned

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Express Mail Receipt No: EL 818 653 469 US

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GREENLEE, WINNER AND SULLIVAN, P.C.
5370 Manhattan Circle, Suite 201
Boulder, CO 80303
Telephone (303) 499-8080
Facsimile: (303) 499-8089
Email: winner@greenwin.com

Attorney docket No. 127-01
bmk: May 9, 2002

10/018418

- 1 -

NOVEL GENES ENCODING WHEAT STARCH SYNTHASES
AND USES THEREFOR

FIELD OF THE INVENTION

5 The present invention relates generally to isolated nucleic acid molecules encoding wheat starch synthase enzymes and more particularly, to isolated nucleic acid molecules that encode wheat SSII and SSIII enzyme activities. The isolated nucleic acid molecules provide the means for modifying starch content and composition in plants, for example the ratio of amylose:amylopectin in the starch granule of the
10 endosperm during the grain-filling phase of endosperm development. The isolated nucleic acid molecules of the present invention also provide the means for screening plant lines to determine the presence of natural and/or induced mutations in starch synthase genes which affect starch content and/or composition. The isolated nucleic acid molecules of the present invention further provide for the screening-assisted
15 breeding of plants having desirable starch content and/or composition, in addition to providing for the direct genetic manipulation of plant starch content and/or composition.

GENERAL

Bibliographic details of the publications numerically referred to in this specification are
20 collected at the end of the description. Reference herein to any published document is not to be taken as an indication or admission that any such published document is part of the common general knowledge or background information of a skilled worker in the relevant field.

25 This specification contains nucleotide and amino acid sequence information (SEQ ID NOS:) prepared using the programme PatentIn Version 2.0, presented herein at the end of the specification. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc)
30 and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>.

- 2 -

respectively. Nucleotide and amino acid sequences (SEQ ID NOs:) referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (eg. SEQ ID NO: 1 is <400>1, etc).

- 5 The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W
10 represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.
- 15 The designations for naturally-occurring amino acid residues referred to herein are set forth in Table I. The designations for a non-limiting set of non-naturally-occurring amino acids is listed in Table 2.

As used herein the term "derived from" shall be taken to indicate that a specified
20 integer may be obtained from a particular source albeit not necessarily directly from that source.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to
25 imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of steps or elements or integers.

- 3 -

TABLE 1

Amino Acid	Three-letter Code	One-letter Code
5 Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
10 Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
15 Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
20 Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
25 Aspartate/glutamate	Baa	B
Asparagine/glutamine		
Any amino acid as above	Xaa	X

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
5 α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino- α -methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
10 aminoisobutyric acid	Aib	L-N-methylaspartic acid	Nmasp
aminonorbornyl- carboxylate	Norb	L-N-methylcysteine	Nmcys
cyclohexylalanine	Chexa	L-N-methylglutamine	Nmgln
cyclopentylalanine	Cpen	L-N-methylglutamic acid	Nmglu
15 D-alanine	Dal	L-N-methylhistidine	Nmhis
D-arginine	Darg	L-N-methylisoleucine	Nmile
D-aspartic acid	Dasp	L-N-methylleucine	Nmleu
D-cysteine	Dcys	L-N-methyllysine	Nmlys
D-glutamine	Dgln	L-N-methylmethionine	Nmmet
20 D-glutamic acid	Dglu	L-N-methylnorleucine	Nmnle
D-histidine	Dhis	L-N-methylnorvaline	Nmnva
D-isoleucine	Dile	L-N-methylornithine	Nmorn
D-leucine	Dleu	L-N-methylphenylalanine	Nmphe
D-lysine	Dlys	L-N-methylproline	Nmpro
25 D-methionine	Dmet	L-N-methylserine	Nmser
D-ornithine	Dorn	L-N-methylthreonine	Nmthr
D-phenylalanine	Dphe	L-N-methyltryptophan	Nmtrp
D-proline	Dpro	L-N-methyltyrosine	Nmtyr
D-serine	Dser	L-N-methylvaline	Nmval
30 D-threonine	Dthr	L-N-methylethylglycine	Nmetg
D-tryptophan	Dtrp	L-N-methyl-t-butylglycine	Nmtbug
		L-norleucine	Nle
		L-norvaline	Nva

	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
5	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- α -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl) glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl) glycine	Nbhe

- 6 -

	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl) glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
5	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl) glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylethyl) glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
10	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
15	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
20	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylasparagine	Masn
	L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
25	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mglu	L- α -methylglutamate	Mglu
	L- α -methylhistidine	Mhis	L- α -methylhomo phenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl) glycine	Nmet
30	L- α -methylleucine	Mleu	L- α -methyllysine	Mlys

- 7 -

L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	Mser	L- α -methylthreonine	Mthr
5 L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
L- α -methylvaline	Mval	L-N-methylhomo	
		phenylalanine	Nmhpe
N-(N-(2,2-diphenylethyl)		N-(N-(3,3-diphenylpropyl)	
carbamylmethyl)glycine	Nnbhm	carbamylmethyl)glycine	Nnbhe
10 1-carboxy-1-(2,2-diphenyl-			
ethylamino)cyclopropane	Nmbc		

Those skilled in the art will appreciate that the invention described herein is susceptible
 15 to variations and modifications other than those specifically described. It is to be
 understood that the invention includes all such variations and modifications. The
 invention also includes all of the steps, features, compositions and compounds
 referred to or indicated in this specification, individually or collectively, and any and all
 combinations or any two or more of said steps or features.

20

The present invention is not to be limited in scope by the specific embodiments
 described herein, which are intended for the purposes of exemplification only.
 Functionally-equivalent products, compositions and methods are clearly within the
 scope of the invention, as described herein.

25

BACKGROUND TO THE INVENTION

The biosynthesis of the starch granule is a complex process which involves the action
 of an array of isoforms of enzymes involved in the starch biosynthesis. Following the
 formation of glucose-1-phosphate, the enzyme activities required for the synthesis of
 30 granular starch include ADP glucose pyrophosphorylase (EC 2.7.7.27), starch
 synthases (EC 2.4.1.21), branching enzymes (EC 2.4.1.18) and debranching enzymes

(EC 3.2.1.41 and EC 3.2.1.68) (Mouille *et al.*, 1996). Plants contain isozymes of each of these activities, and the definition of these isoforms and their roles has been conducted through investigation of the properties of the suite of soluble enzymes found in the stroma of the plastid, analysis of the proteins entrapped within the matrix of the starch granule, and mutational studies to identify genes and define linkages between individual genes and their specific roles.

Starch synthases extend regions of α -1,4 glucan through the transfer of the glucosyl moiety of ADPglucose to the non-reducing end of a pre-existing α -1,4 glucan. In addition to GBSS, 3 other classes of starch synthase have been identified in plants, SSI (wheat, Li *et al.*, 1999 and GenBank Accession No. U48227; rice, Baba *et al.*, 1993; potato, Genbank Accession No. STSTASYNT), SSII (pea, Dry *et al.* 1992; potato, Edwards *et al.*, 1995; maize, Harn *et al.* 1998 and GenBank Accession No. U66377) and SSIII (potato, Abel *et al.*, 1996; maize, Gao *et al.*, 1998). In the cereals, the most comprehensively studied species is maize, where in addition to GBSS, cDNAs encoding SSI, SSIIa, and SSIIb have been isolated, and both cDNA and genomic clones for *dull1* have been characterised (Knight *et al.*, 1998; Harn *et al.*, 1998; Gao *et al.*, 1998). In maize, the product of the *du1* gene is known as maize SSII, however this gene is the homologue of potato SSIII.

20

The proteins within the matrix of the wheat starch granule have been extensively studied (Denyer *et al.*, 1995; Rahman *et al.*, 1995; Takaoka *et al.*, 1997; Yamamori and Endo, 1996) and 60, 75, 85, 100, 104 and 105 kDa protein bands can be visualised following SDS-PAGE. The predominant 60 kDa protein is exclusively granule-bound and is analogous to the "waxy" granule bound starch synthase (GBSS) gene in maize (Rahman *et al.*, 1995). The combination of three null alleles for this enzyme from each of the wheat genomes (Nakamura *et al.*, 1995) results in the amylose-free "waxy" phenotype found in other species. The 75 kDa starch synthase I (wSSI) is found in both the granule and the soluble fraction of wheat endosperm (Denyer *et al.*, 1995; Li *et al.*, 1999) and has been assigned to chromosomes 7A, 7B and 7D (Yamamori and Endo, 1996; Li *et al.*, 1999). The 85 kDa band contains a

30

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class II branching enzyme and an unidentified polypeptide (Rahman *et al.*, 1995). The 100, 104 and 105 kDa proteins of the wheat starch granule (designated Sgp-B1, Sgp-D1 and Sgp-A1 by Yamamori and Endo, 1996) have been shown to be encoded by a homeologous set of genes on the short arm of chromosome 7B, 7A and 7D 5 respectively (Yamamori and Endo, 1996; Takaoka *et al.*, 1997). Denyer *et al.* (1995) concluded on the basis of enzyme activity assays that these proteins were also starch synthases. These genes are referred to hereinafter as the "wheat SSII genes".

While GBSS has been established to be essential for amylose synthesis, the remaining 10 starch synthases are thought to be primarily responsible for the elongation of amylopectin chains, although this does not preclude them from also having non-essential roles in amylose biosynthesis. Differences in kinetic properties between isoforms, and the analysis of mutants lacking various isoforms, suggests that each isoenzyme contributes to the extension of specific subsets of the available non- 15 reducing ends.

SUMMARY OF THE INVENTION

The production of plants that produce improved starches that are modified for particular end-use applications, such as, for example, starches having high or low 20 amylose:amylopectin ratios, requires the availability of genes encoding the various starch synthase isoforms. Because of species-specific codon usages, and variations in the kinetic parameters of the starch synthase isoforms between species, the production of modified starches may require the use of genes derived from particular species.

25

Furthermore, the screening-assisted breeding of plants having desirable starch content and/or composition requires specific gene sequences to be provided that can be used to distinguish between different homeologous genes encoding the various isoforms of wheat starch synthases, such as, for example, to identify and distinguish between 30 naturally-occurring variant gene sequences. It is a particular object of the present invention to provide gene sequences to facilitate the screening-assisted selection of

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wheat plants having starch traits which are associated with the presence and/or expression of one or more wheat SSI and/or SSIII genes.

Accordingly, the present invention provides isolated nucleotide sequences encoding
5 the wheat SSII (i.e. wSSII) and wheat SSIII (i.e. wSSIII) isoenzymes, and DNA markers derived therefrom. The present invention further facilitates the production of transformed plants carrying these nucleotide sequences.

More particularly, the present invention provides isolated nucleic acid molecules
10 encoding the 100, 104 and 105 kDa SSII (Sgp-1) polypeptides of the wheat starch granule matrix, as determined using the SDS/PAGE system of Rahman *et al.* (1995), which polypeptides are equivalent to the 100, 108 and 115 kDa polypeptides described by Yamamori and Endo (1996).

15 The present invention further provides isolated nucleic acid molecules encoding the soluble *dull1*-type wheat starch synthase III polypeptide. Analysis of the polypeptides encoded by these nucleic acid molecules reveals several consensus amino acid sequence motifs that are highly conserved in wheat starch synthase isoenzymes, in addition to isoenzyme-specific sequences, which sequences possess utility in isolating
20 related starch synthase-encoding sequences and in assaying plants for their expression of one or more starch synthase isoenzymes.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides which encodes, or is
25 complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof selected from the following:

- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at
30 least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;

(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;

5 (iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- 10 (a) KVGGLGDVVTS (SEQ ID NO: 39);
- (b) GHTVEVILPKY (SEQ ID NO: 40);
- (c) HDWSSAPVAWLYKEHY (SEQ ID NO: 41);
- (d) GILNGIDPDIWDPYTD (SEQ ID NO: 42);
- (e) DVPIVGIIITRLTAQKG (SEQ ID NO: 43);
- (f) NGQVVLLGSA (SEQ ID NO: 44);
- 15 (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS (SEQ ID NO: 45); and
- (h) TGGLVDTV (SEQ ID NO: 46);

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

20 (iv) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- 25 (a) KTGGLGDVAGA (SEQ ID NO: 47);
- (b) GHRVMVVVPRY (SEQ ID NO: 48);
- (c) NDWHTALLPVYLKAYY (SEQ ID NO: 49);
- (d) GIVNGIDNMEWNPEVD (SEQ ID NO: 50);
- (e) DVPLLGFIGRLDGQKG (SEQ ID NO: 51);
- (f) DVQLVMLGTG (SEQ ID NO: 52);
- 30 (g) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO: 53); and
- (h) VGG(V/L)RDTV (SEQ ID NO: 54);

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wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

5 In a preferred embodiment, the isolated nucleic acid molecule encodes a starch synthase polypeptide, protein or enzyme having at least about 90% amino acid sequence identity to any one of SEQ ID NOS: 2, 4, 6, 8 or 10, more preferably having at least about 95% or about 97% or about 99% identity to any one of said amino acid sequences.

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In an alternative embodiment, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:

- (a) GHTVEVILPKY;
- 15 (b) HDWSSAPVAWLYKEHY;
- (c) DVPIVGIIITRLTAQKG;
- (d) NGQVVLLGSA;
- (e) AGSDFIIVPSIFPCGLTQLVAMRYGS;
- (f) TGGLVDTV;
- 20 (g) GIVNGIDNMEWNPEVD; and
- (h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.

in an alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme
25 molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence having at least about 85% nucleotide sequence identity to any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37 or 38 or a complementary nucleotide sequence thereto.

30 In a preferred embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37 or 38,

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or is at least about 90% identical, more preferably at least about 95% or 97% or 99% identical to all or a protein-encoding part thereof.

In an alternative embodiment, the present invention provides an isolated nucleic acid
5 molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence that is capable of hybridising under at least moderate stringency hybridisation conditions to at least about 30 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary
10 nucleotide sequence thereto.

A second aspect of the present invention provides a method of isolating a nucleic acid molecule that encodes a starch synthase polypeptide, protein or enzyme described *supra*, said method comprising:

- 15 (i) hybridising a probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto to single-stranded or double-stranded mRNA, cDNA or genomic DNA; and
(ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting
20 means.

Preferably, the detecting means is a reporter molecule covalently attached to the probe or primer molecule or alternatively, a polymerase chain reaction format. Accordingly, the present invention clearly extends to the use of the nucleic acid molecules provided
25 herein to isolate related starch synthase-encoding sequences using standard hybridisation and/or polymerase chain reaction techniques.

A third aspect of the invention provides an isolated probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3,
30 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto.

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Preferably, the probe or primer comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 25 to 34.

A fourth aspect of the present invention is directed to an isolated or recombinant starch
5 synthase polypeptide, protein or enzyme, preferably substantially free of conspecific or non-specific proteins, which comprises an amino acid sequence selected from the following:

- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at
10 least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;
- (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at
15 least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;
- (iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:
 - (a) KVGGLGDVVTs;
 - (b) GHTVEVILPKY;
 - (c) HDWSSAPVAWLYKEHY;
 - (d) GILNGIDPDIWDPYTD;
 - (e) DVPIVGIITRLTAQKG;
 - (f) NGQVVLLGSA;
 - (g) AGSDFIIVPSIFPCGLTQLVAMRYGS; and
 - (h) TGGLVDTV

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid
30 sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

- (iv) a wheat starch synthase polypeptide, protein or enzyme or functional

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subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (a) KTGGLGDVAGA;
- 5 (b) GHRVMVVVPRY;
- (c) NDWHTALLPVYLKAYY;
- (d) GIVNGIDNMEWNPEVD;
- (e) DVPLLGFGRDLGQKG;
- (f) DVQLVMLGTG;
- 10 (g) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (h) VGG(V/L)RDTV

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

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The present invention clearly encompasses the mature protein region of a wheat starch synthase polypeptide which is obtained by removal of the N-terminal transit peptide sequence.

- 20 A further aspect of the invention provides a method of assaying for the presence or absence of a starch synthase isoenzyme or the copy number of a gene encoding same in a plant, comprising contacting a biological sample derived from said plant with an isolated nucleic acid molecule derived from any one of SEQ ID NOS 1, 3, 5, 7, 9, 11-16, 37 or 38, or any one of SEQ ID NOS: 25 to 34, or a complementary nucleotide
- 25 sequence thereto for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means.

The detection means according to this aspect of the invention is any nucleic acid based hybridisation or amplification reaction.

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A further aspect of the present invention utilises the above-mentioned assay method

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in the breeding and/or selection of plants which express or do not express particular starch synthase isoenzymes or alternatively, which express a particular starch synthase isoenzyme at a particular level in one or more plant tissues. This aspect clearly extends to the selection of transformed plant material which contains one or
5 more of the isolated nucleic acid molecules of the present invention.

A further aspect of the present invention provides a method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing therein a sense molecule, antisense molecule, ribozyme
10 molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified. This aspect of the invention clearly extends to the
15 introduction of the sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule to isolated plant cells, tissues or organs or organelles by cell fusion or transgenic means and the regeneration of intact plants therefrom.

20 A further aspect of the present invention provides an isolated promoter that is operable in the endosperm of a monocotyledonous plant cell, tissue or organ, and preferably in the endosperm of a monocotyledonous plant cell, tissue or organ. For example, the HMG promoter from wheat, or the maize zein gene promoter are particularly preferred, as is the promoter derived from a starch synthase gene of the present invention, such
25 as a promoter that is linked *in vivo* to any one of SEQ ID NOS 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto.

A still further aspect of the present invention contemplates a transgenic plant comprising an introduced sense molecule, antisense molecule, ribozyme molecule, co-
30 suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16,

37 or 38, or a complementary nucleotide sequence thereto or a genetic construct comprising same, and to plant propagules, cells, tissues, organs or plant parts derived from said transgenic plant that also carry the introduced molecule(s).

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a copy of a photographic representation showing the distribution of wheat endosperm starch synthases between the starch granule and soluble fractions. Lane 1, SDS-PAGE of wheat endosperm starch granule proteins revealed by silver staining; lanes 2-7, immunoblot of wheat endosperm soluble phase and starch granule proteins separated by SDS-PAGE from various developmental stages and probed with an anti- (wheat wSSII peptide) monoclonal antibody. Lanes 2-4 contain proteins from the soluble fraction of wheat endosperm at 15 days post anthesis (Lane 2); 20 days post anthesis (Lane 3); and at 25 days post anthesis (Lane 4). Lanes 5-7 contain proteins from the starch granule of wheat endosperm at 15 days post anthesis (Lane 5); 20 days post anthesis (Lane 6); and at 25 days post anthesis (Lane 7).

Figure 2 is a copy of a schematic representation comparing the nucleotide sequences of cDNA clones designated wSSIIA, wSSIIb and wSSIID, encoding the starch synthase II polypeptides from wheat, using the PILEUP programme of Devereaux *et al.* (1984).

Figure 3 is a copy of a schematic representation comparing the deduced amino acid sequences of starch synthase II from wheat (wSSIIA, wSSIIb and wSSIID), maize (maize SSIIa and maize SSIIb; Harn *et al.*, 1998), pea (pea SSII; Dry *et al.*, 1992) and potato (potato SSII; van der Leij *et al.*, 1991). Identical amino acid residues among each of these sequences are indicated below the sequences with "*". The alignments of maize SSIIa with maize SSIIb, and pea SSII and potato SSII are essentially as described in Harn *et al.* (1998) and Edwards *et al.* (1995). All sequences are aligned to position the transit peptide cleavage site below the arrow (↓) between residues 59 and 60 of the wSSIIA sequence. The wSSIIp1 sequence, the sequence of SGP-B1 (peptide3), and of eight conserved regions are annotated and underlined.

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Figure 4 is a copy of a photographic representation of a northern blot showing the expression of wheat wSSII mRNA in wheat plants. Total RNAs were isolated from leaves pre-anthesis florets and endosperm of the wheat cultivar "Gabo", grown under a photoperiod comprising 16 hours daylength, and at 18 °C during the day, and at 13 °C during the night cycle, and probed with the wSSIIp2 DNA fragment. The source of each RNA is indicated at the top of the Figure as follows: Lane 1, leaf; Lane 2, pre-anthesis florets; Lanes 3-11, endosperm at: 4 days post-anthesis (Lane 3); 6 days post-anthesis (Lane 4); 8 days post-anthesis (Lane 5); 10 days post-anthesis (Lane 6); 12 days post-anthesis (Lane 7); 15 days post-anthesis (Lane 8); 18 days post-anthesis (Lane 9); 21 days post-anthesis (Lane 10); and 25 days post-anthesis (Lane 11).

Figure 5 is a copy of a photographic representation showing the localization of wheat starch synthase II genes on the wheat genome by PCR, using the primers ssIIc, ssIIId and ssIIe in the amplification reaction. The nullisomic-tetrasomic genomic DNA of wheat cv. Chinese Spring was used as template DNA. Lane D, *Triticum tauschii*; Lane AB, Accession line N7DT7B having no 7D chromosome and four copies of the 7B chromosome; Lane AD, Accession line N7BT7A having no 7B chromosome and four copies of the 7A chromosome; Lane BD, Accession line N7AT7B having no 7A chromosome and four copies of the 7B chromosome; Lane ABD, wheat cv. Chinese Spring. PCR products derived from each cDNA clone are labelled. The results indicate that the cDNA clones, wSSIIB, wSSIIA and wSSIID are derived from the B-, A- and D-genomes of wheat, respectively.

Figure 6 is a schematic representation showing the organisation of introns (lines) and exons (boxes) in the wheat SSII gene shown in SEQ ID NO: 37. The scale (bases), relative to the nucleotide sequence set forth in SEQ ID NO: 37, is provided at the bottom of the figure.

Figure 7 is a schematic representation comparing the deduced amino acid Sequences of the maize, potato and wheat SSIII polypeptides.

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Figure 8 is a copy of a photographic representation showing the expression of wheat wSSIII mRNA in wheat. Total RNAs were isolated from the endosperm of the wheat cultivars Wyuna (Panel a) and Gabo (Panel b) leaves pre-anthesis florets and endosperm of the wheat cultivar "Gabo", grown under a photoperiod comprising 16 hours daylength, and at 18 °C during the day cycle, and at 13 °C during the night cycle, and probed with the wSSIIIp1 DNA fragment derived from wSSIII.B3 cDNA. The source of each RNA is indicated at the top of the Figure as follows: Lane 1, endosperm at: 4 days post-anthesis; Lane 2, endosperm at 6 days post-anthesis; Lane 4, endosperm at 8 days post-anthesis; Lane 4, endosperm at 10 days post-anthesis; 10 Lane 5, endosperm at 12 days post-anthesis; Lane 6, endosperm at 15 days post-anthesis; Lane 7, endosperm at 18 days post-anthesis; Lane 8, endosperm at 21 days post-anthesis; Lane 9, endosperm at 25 days post-anthesis; and Lane 10, endosperm at 31 days post-anthesis (Panel a only). In panel (c), L refers to leaf RNA, and P refers to RNA from pre-anthesis florets derived from the cultivar Gabo.

15

Figure 9 is a schematic representation showing the position of conserved amino acid sequences within four wheat starch synthase proteins. The eight highly-conserved regions between the wheat starch synthase polypeptides are underlined and annotated at the top of each group of amino acid sequences. The sequences included in the 20 alignment are the wheat SSII-A1 and wheat SSIII polypeptides of the present invention; wheat GBSS (wGBSS; Yan *et al.*, 1999); wheat SSI (wSS1; Li *et al.*, 1999); wheat SSII (wSS2; SEQ ID NO: 4); and wheat SSIII (wSS3; SEQ ID NO: 8).

Figure 10 is a schematic representation showing the relationships between the 25 primary amino acid sequences of starch synthases (SS) and glycogen synthase of *E. coli* (GS). The dendrogram was generated by the program PILEUP (Devereaux *et al.*, 1984). The amino acid sequences used for the analysis are those of the wheat SSIIA, wheat SSIIIB, wheat SSIIID, and wheat SSIII polypeptides of the present invention compared to the deduced amino acid sequences of wheat GBSS (Clark *et al.*, 1991), 30 wheat SSI (Li *et al.*, 1999), rice GBSS (Okagaki, 1992), rice SSI (Baba *et al.*, 1993), maize GBSS (Kloesgen *et al.*, 1986), maize SSI (Knight *et al.*, 1998), maize SSIIa and

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maize SSIIb (Harn *et al.*, 1998), maize SSIII (Gao *et al.*, 1998), pea GBSS (Dry *et al.*, 1992), pea SSII (Dry *et al.*, 1992), potato GBSS (van der Leij *et al.*, 1991), potato SSI (Genbank accession number: STSTASYNT), potato SSII (Edwards *et al.*, 1995), potato SSIII (Abel *et al.*, 1996), and *E. coli* glycogen synthase (GS) (Kumar *et al.*, 1986). Five groups of enzymes included in the alignment are granule-bound starch synthase (GBSS), starch synthase-I (SSI), starch synthase-II (SSII), starch synthase-III (SSIII) and glycogen synthase (GS).

Figure 11 is a schematic representation showing the position of conserved regions within cereal starch synthase genes. Comparisons of cereal starch synthases were made based on their deduced amino acid sequences and 8 conserved regions identified. Conserved regions are shown in bold and transit peptides (where defined) in grey. The sequences included in the alignment are the wheat SSII-A1 and wheat SSIII polypeptides of the present invention; wheat GBSS (Ainsworth *et al.*, 1993); wheat SSI (Li *et al.*, 1999); maize SSIIa (Harn *et al.*, 1998); and maize dull-1 (Gao *et al.*, 1998).

Figure 12 is a copy of a schematic representation of a gene map showing the alignment of fragments 1 to 6 of the genomic SSIII gene (lower line) with the corresponding SSIII cDNA clone (upper line). Raised regions in the genomic clone fragments (lower line) represent protein-encoding regions of the gene.

Figure 13 is a schematic representation showing the organisation of introns (lines) and exons (boxes) in the wheat SSIII gene shown in SEQ ID NO: 38. The scale (bases), relative to the nucleotide sequence set forth in SEQ ID NO: 38, is provided at the bottom of the figure.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides which encodes, or is complementary to a nucleic

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acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof selected from the following:

- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6; and
- (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10.

10 Alternatively or in addition, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof and comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, or 37.

15 Alternatively or in addition, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof and comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 7, 9, or 38.

20 As used herein, the term "starch synthase" shall be taken to refer to any enzymatically-active peptide, polypeptide, oligopeptide, polypeptide, protein or enzyme molecule that is at least capable of transferring a glucosyl moiety from ADP-glucose to an α -1,4-glucan molecule, or a peptide, polypeptide, oligopeptide or polypeptide fragment of such an enzymatically-active molecule.

25

The term "wheat starch synthase" refers to a starch synthase derived from hexaploid wheat or barley or a progenitor species, or a relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others, the only requirement that the genomic DNA is at least about 80% identical to the genome of a wheat plant as determined by standard DNA melting curve analyses.

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The term "starch synthase II" or "wSSII" or similar term shall be taken to refer to a starch synthase as hereinbefore defined that is detectable in the starch granule of a plant seed endosperm and possesses one or more properties selected from the group consisting of:

- 5 (i) it is immunologically cross-reactive with the wheat starch granule proteins designated Sgp-B1 and/or Sgp-D1 and/or Sgp-A1, having estimated molecular weights of about 85 kDa to about 115 kDa;
- (ii) it is encoded by one of a homeologous set of genes localised on wheat chromosomes 7B or 7A or 7D;
- 10 (iii) it is encoded by a nucleotide sequence that comprises at least about 15 nucleotides in length derived from any one or more of SEQ ID NOS: 1, 3, 5, or 37 or a complementary nucleotide sequence thereto;
- (iv) it is encoded by a nucleotide sequence that is at least about 85% identical to one or more of the nucleotide sequences set forth in SEQ ID NOS:
- 15 1, 3, 5, or 37, or a complementary nucleotide sequence thereto;
- (v) it comprises an amino acid sequence having at least about 85% identity to one or more of SEQ ID NOS: 2 or 4 or 6;
- (vi) it comprises at least about 5 contiguous amino acids, preferably at least about 10 contiguous amino acids, more preferably at least about 15 contiguous amino acids, even more preferably at least about 20 contiguous amino acids
- 20 and still even more preferably at least about 25-50 contiguous amino acids of the amino acid sequences set forth in SEQ ID NOS: 2 or 4 or 6;
- (vii) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:
- 25 (a) KVGGLGDVVTS;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIIITRLTAQKG;
- 30 (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFPCGLTQLVAMRYGS; and

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(h)TGGLVDTV,

in addition to any one or more of (i) to (vi); and

(viii) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- 5 (a) KTGGLGDVAGA;
- (b) GHRVMVVVPRY;
- (c) NDWHTALLPVYLKAYY;
- (d) GIVNGIDNMEWNPEVD;
- (e) DVPLLGFIGRLDGQKG;
- 10 (f) DVQLVMLGTG;
- (g)AGADALLMPSTRF(E/V)PCGLNQLYAMAYGT; and
- (h)VGG(V/L)RDTV,

in addition to any one or more of (i) to (vi).

15 The term "starch synthase III" or "wSSIII" or similar term shall be taken to refer to a starch synthase as hereinbefore defined that possesses one or more properties selected from the group consisting of:

- (i) it is encoded by a nucleotide sequence that comprises at least about 15 nucleotides in length derived from any one or more of SEQ ID NOS: 7, 9, 11-20 16, or 38, or a complementary nucleotide sequence thereto;
- (ii) it is encoded by a nucleotide sequence that is at least about 85% identical to one or more of the nucleotide sequences set forth in SEQ ID NOS: 7, 9, 11-16, or 38, or a complementary nucleotide sequence thereto; and
- (iii) it comprises an amino acid sequence having at least about 85% identity25 to one or more of SEQ ID NOS: 8 or 10;
- (iv) it comprises at least about 5 contiguous amino acids, preferably at least about 10 contiguous amino acids, more preferably at least about 15 contiguous amino acids, even more preferably at least about 20 contiguous amino acids and still even more preferably at least about 25-50 contiguous amino acids of30 the amino acid sequences set forth in SEQ ID NOS: 8 or 10;
- (v) which comprises a conserved amino acid sequence having at least 25%

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identity to an amino acid sequence selected from the group consisting of:

- (a) KVGGLGDVVTS;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIIITRLTAQKG;
- (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFEPGLTQLVAMRYGS; and
- (h) TGGLVDTV

in addition to any one or more of (i) to (iv); and

(vi) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (a) KTGGLGDVAGA;
- (b) GHRVMVVVPRY;
- (c) NDWHTALLPVYLKAYY;
- (d) GIVNGIDNMEWNPEVD;
- (e) DVPLLGFIRLDGQKG;
- (f) DVQLVMLGTG;
- (g) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (h) VGG(V/L)RDTV,

in addition to any one or more of (i) to (iv).

In a more preferred embodiment, the WSSII or WSSIII polypeptide encoded by the nucleic acid molecule of the present invention will comprise a substantial contiguous region of any one of SEQ ID NOS: 2, 4, 6, 8 or 10 or 17 sufficient to possess the biological activity of a starch synthase polypeptide.

For the purposes of nomenclature, the nucleotide sequence set forth in SEQ ID NO: 1 relates to the cDNA molecule encoding the WSSII (i.e. Sgp-B1) polypeptide of wheat. The amino acid sequence of the corresponding polypeptide is set forth herein as SEQ ID NO:2. The nucleotide sequence set forth in SEQ ID NO: 3 relates to the

Preferably, the isolated nucleic acid molecule of the present invention comprises a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8, or 10 and more preferably, which additionally comprises which comprises one or more amino acid sequences selected from the group consisting of:

- 25 (a) KVGGLGDVVTS;
(b) GHTVEVILPKY;
(c) HDWSSAPVAWLYKEHY;
(d) GILNGIDPDIWDPYTD;
(e) DVPIVGIIITRLTAQKG;
30 (f) NGQVVLLGSA;
(g) AGSDFIIVPSIFEPGLTQLVAMRYGS;

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- (h)TGGLVDTV;
- (i) KTGGLGDVAGA;
- (j) GHRVMVVVPRY;
- (k) NDWHTALLPVYLKAYY;
- 5 (l) GIVNGIDNMEWNPEVD;
- (m) DVPLLGFIGRLDGQKG;
- (n) DVQLVMLGTG;
- (o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (p)VGG(V/L)RDTV.

10

The present invention clearly extends to homologues, analogues and derivatives of the wheat starch synthase II and III genes exemplified by the nucleotide sequences set forth herein as SEQ ID NOs: 1, 3, 5, 7, 9, 11-16, 37 or 38.

15 Preferred starch synthase genes may be derived from a naturally-occurring starch synthase gene by standard recombinant techniques. Generally, a starch synthase gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the starch synthase gene of the present invention include 5' and 3' terminal fusions as
20 well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the
25 sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or
30 hydrophobicity.

For the present purpose, "homologues" of a nucleotide sequence shall be taken to

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refer to an isolated nucleic acid molecule which is substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence within said sequence, of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

5

"Analogues" of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence of any non-nucleotide constituents not normally
10 present in said isolated nucleic acid molecule, for example carbohydrates, radiochemicals including radionucleotides, reporter molecules such as, but not limited to DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

"Derivatives" of a nucleotide sequence set forth herein shall be taken to refer to any
15 isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5' and 3' terminal fusions as well
20 as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of said sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional variants are
25 characterised by the removal of one or more nucleotides from the nucleotide sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

30 The present invention extends to the isolated nucleic acid molecule when integrated into the genome of a cell as an addition to the endogenous cellular complement of starch synthase genes, irrespective of whether or not the introduced nucleotide

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sequence is translatable or non-translatable to produce a polypeptide. The present invention clearly contemplates the introduction of additional copies of starch synthase genes into plants, particularly wheat plants, in the antisense orientation to reduce the expression of particular wheat starch synthase genes. As will be known to those skilled
5 in the art, such antisense genes are non-translatable, notwithstanding that they can be expressed to produce antisense mRNA molecules.

The said integrated nucleic acid molecule may, or may not, contain promoter sequences to regulate expression of the subject genetic sequence.

10

Accordingly, the present invention clearly encompasses preferred homologues, analogues and derivatives that comprise a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof

15 selected from the following:

- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;
- 20 (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;
- (iii) a wheat starch synthase polypeptide, protein or enzyme or functional
25 subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:
 - (a) KVGGLGDVVTs;
 - (b) GHTVEVILPKY;
 - 30 (c) HDWSSAPVAWLYKEHY;
 - (d) GILNGIDPDIWDPYTD;

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- (e) DVPIVGIIITRLTAQKG;
- (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFEPGLTQLVAMRYGS; and
- (h) TGGLVDTV

5 and wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

(iv) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at

10 least 25% identity to an amino acid sequence selected from the group consisting of:

- (a) KTGGLGDVAGA;
- (b) GHRVMVVVPRY;
- (c) NDWHTALLPVYLKAYY;
- 15 (d) GIVNGIDNMEWNPEVD;
- (e) DVPLLGFGRDLGQKG;
- (f) DVQLVMLGTG;
- (g) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (h) VGG(V/L)RDTV,

20 and wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

Preferably, the isolated nucleic acid molecule encodes a starch synthase polypeptide,

25 protein or enzyme that comprises two, more preferably three, more preferably four, more preferably five, more preferably six, more preferably seven and even more preferably eight of the conserved amino acid motifs listed *supra*. Even more preferably, the said amino acid motifs are located in a relative configuration such as that shown for the wheat SSII or wheat SSIII polypeptides described herein.

30

In a preferred embodiment, the isolated nucleic acid molecule encodes a starch

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synthase polypeptide, protein or enzyme having at least about 90% amino acid sequence identity to any one of SEQ ID NOS: 2, 4, 6, 8 or 10, more preferably having at least about 95% or about 97% or about 99% identity to any one of said amino acid sequences.

5

In an alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence having at least about 85% nucleotide sequence identity to any
10 one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a degenerate nucleotide sequence thereto or a complementary nucleotide sequence thereto.

By "degenerate nucleotide sequence" is meant a nucleotide sequence that encodes a substantially identical amino acid sequence as a stated nucleotide sequence.

15

In a preferred embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or is at least about 90% identical, more preferably at least about 95% or 97% or 99% identical to all or a protein-encoding part thereof.

20

In an alternative embodiment, preferred homologues, analogues and derivatives of the nucleic acid molecule of the present invention encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof and comprises a nucleotide sequence that is capable of hybridising under at least moderate
25 stringency hybridisation conditions to at least about 30 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto.

For the purposes of defining the level of stringency, a low stringency is defined herein
30 as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. Generally, the stringency is increased by reducing the concentration of SSC

buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. A moderate stringency comprises a hybridisation and/or a wash carried out in 0.2 x SSC-2 x SSC buffer, 0.1% (w/v) SDS at 42°C to 65°C, while a high stringency comprises a hybridisation and/or a wash carried out in
5 0.1xSSC-0.2 x SSC buffer, 0.1% (w/v) SDS at a temperature of at least 55°C. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of further clarification only, reference to the parameters affecting hybridisation between nucleic acid molecules is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

10

Those skilled in the art will be aware of procedures for the isolation of further wheat starch synthase genes to those specifically described herein or homologues, analogues or derivatives of said genes, for example further cDNA sequences and genomic gene equivalents, when provided with one or more of the nucleotide
15 sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 9,11-16, 37, or 38. In particular, amplifications and/or hybridisations may be performed using one or more nucleic acid primers or hybridisation probes comprising at least 10 contiguous nucleotides and preferably at least about 20 contiguous nucleotides or 50 contiguous nucleotides derived from the nucleotide sequences set forth herein, to isolate cDNA clones, mRNA
20 molecules, genomic clones from a genomic library (in particular genomic clones containing the entire 5' upstream region of the gene including the promoter sequence, and the entire coding region and 3'-untranslated sequences), and/or synthetic oligonucleotide molecules, amongst others. The present invention clearly extends to such related sequences.

25

Accordingly, a second aspect of the present invention provides a method of isolating a nucleic acid molecule that encodes a starch synthase polypeptide, protein or enzyme said method comprising:

- (i) hybridising a probe or primer comprising at least about 15 contiguous
30 nucleotides in length derived from any one of SEQ ID NOS 1, 3, 5, 7, 9,11-16, 37, or 38, or a complementary nucleotide sequence thereto to single-stranded or double-stranded mRNA, cDNA or genomic DNA; and

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- (ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.

Preferably, the detecting means is a reporter molecule covalently attached to the probe
5 or primer molecule or alternatively, a polymerase chain reaction format.

An alternative method contemplated in the present invention involves hybridising two nucleic acid "primer molecules" to a nucleic acid "template molecule" which comprises a related starch synthase gene or related starch synthase genetic sequence or a
10 functional part thereof, wherein the first of said primers comprises contiguous nucleotides derived from any one or more of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, and the second of said primers comprises contiguous nucleotides complementary to any one or more of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38. Specific nucleic acid molecule copies of the template molecule are amplified enzymatically in a
15 polymerase chain reaction, a technique that is well known to one skilled in the art.

In a preferred embodiment, each nucleic acid primer molecule is at least 10 nucleotides in length, more preferably at least 20 nucleotides in length, even more preferably at least 30 nucleotides in length, still more preferably at least 40 nucleotides
20 in length and even still more preferably at least 50 nucleotides in length.

Furthermore, the nucleic acid primer molecules consists of a combination of any of the nucleotides adenine, cytidine, guanine, thymidine, or inosine, or functional analogues or derivatives thereof which are at least capable of being incorporated into a
25 polynucleotide molecule without having an inhibitory effect on the hybridisation of said primer to the template molecule in the environment in which it is used.

Furthermore, one or both of the nucleic acid primer molecules may be contained in an aqueous mixture of other nucleic acid primer molecules, for example a mixture of
30 degenerate primer sequences which vary from each other by one or more nucleotide substitutions or deletions. Alternatively, one or both of the nucleic acid primer molecules may be in a substantially pure form.

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The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the nucleic acid template molecule is derived from a plant cell, tissue or organ, in particular a cell, tissue or organ derived from a wheat or barley plant or a progenitor species, or a
5 relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others.

Those skilled in the art will be aware that there are many known variations of the basic polymerase chain reaction procedure, which may be employed to isolate a related
10 starch synthase gene or related starch synthase genetic sequence when provided with the nucleotide sequences set forth herein. Such variations are discussed, for example, in McPherson *et al* (1991). The present invention extends to the use of all such variations in the isolation of related starch synthase genes or related starch synthase genetic sequences using the nucleotide sequences embodied by the present invention.
15

As exemplified herein, the present inventors have isolated several wheat starch synthase genes using both hybridisation and polymerase chain reaction approaches, employing novel probes and primer sequences to do so.

20 Accordingly, a third aspect of the invention provides an isolated probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto.

25 Preferably, the probe or primer comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 25 to 34.

The isolated nucleic acid molecule of the present invention may be introduced into and expressed in any cell, for example a plant cell, fungal cell, insect cell, animal cell, yeast
30 cell or bacterial cell. Those skilled in the art will be aware of any modifications which are required to the codon usage or promoter sequences or other regulatory

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sequences, in order for expression to occur in such cells.

A further aspect of the invention provides a method of assaying for the presence or absence of a starch synthase isoenzyme or the copy number of a gene encoding same
5 in a plant, comprising contacting a biological sample derived from said plant with an isolated nucleic acid molecule derived from any one of SEQ ID NOS 1, 3, 5, 7, 9, 11-16, 37, or 38, or any one of SEQ ID NOS: 25 to 34, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means.

10

The detection means according to this aspect of the invention is any nucleic acid based hybridisation or amplification reaction.

The hexaploid nature of wheat prevents the straightforward identification of starch
15 synthase allelic variants by hybridisation using the complete starch synthase-encoding sequence, because the similarities between the various alleles generally results in significant cross-hybridisation. Accordingly, sequence-specific hybridisation probes are required to distinguish between the various alleles. Similarly, wherein PCR is used to amplify specific allelic variants of a starch synthase gene, one or more sequence-
20 specific amplification primers are generally required. As will be apparent from the amino acid sequence comparisons provided herein, such as in Figures 3 and 13, non-conserved regions of particular wheat starch synthase polypeptides are particularly useful for the design of probes and primers that are capable of distinguishing between one or more starch synthase polypeptide isoenzyme or allelic variant. The present
25 invention clearly contemplates the design of such probes and primers based upon the sequence comparisons provided herein.

In the performance of this embodiment of the present invention, the present inventors particularly contemplate the identification of wheat starch synthase null alleles or
30 alternatively, mutations wherein specific amino acids are inserted or deleted or substituted, compared to one or more of the wheat SSII or SSIII alleles disclosed

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herein. Such null alleles and other allelic variants are readily identifiable using PCR screening which employs amplification primers based upon the nucleotide and amino acid sequences disclosed herein for SSII and/or SSIII. Once identified, the various mutations can be stacked or pyramided into one or more new wheat lines, such as by
5 introgression and/or standard plant breeding and/or recombinant approaches (eg. transformation, transfection, etc) thereby producing a novel germplasm which exhibits altered starch properties compared to existing lines. DNA markers based upon the nucleotide and amino acid sequences disclosed herein for SSII and/or SSIII can be employed to monitor the stacking of genes into the new lines and to correlate the
10 presence of particular genes with starch phenotypes of said lines.

In this regard, a significant advantage conferred by the present invention is the design of new DNA markers that reveal polymorphisms such as, for example, length polymorphisms, restriction site polymorphisms, and single nucleotide polymorphisms,
15 amongst others, between wheat starch synthases and, in particular, between wheat GBSS and/or SSI and/or SSII and/or SSIII, or between allelic variants of one or more of said starch synthases, that can be used to identify the three genomes of hexaploid wheats (i.e., the A, B and D genomes).

20 Preferably, such DNA markers are derived from the intron region of a starch synthase gene disclosed herein, more preferably the wheat SSII and/or the wheat SSIII gene. Those skilled in the art will be aware that such regions generally have a higher degree of variation than in the protein-encoding regions and, as a consequence, are particularly useful in identifying specific allelic variants of a particular gene, such as
25 allelic variants contained in any one of the three wheat genomes, or alternatively or in addition, for the purpose of distinguishing between wheat GBSS, SSI, SSII or SSIII genes.

A further approach contemplated by the present inventors is the design of unique
30 isoenzyme-specific and/or allele-specific peptides based upon the amino acid sequence disclosed herein as SEQ ID NOS: 25 and/or SEQ ID NO: 4 and/or SEQ ID

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NO: 6 and/or SEQ ID NO: 8 and/or SEQ ID NO: 10, which peptides are then used to produce polyclonal or monoclonal antibodies by conventional means. Alternatively, the genes encoding these polypeptides or unique peptide regions thereof can be introduced in an expressible format into an appropriate prokaryotic or eukaryotic
5 expression system, where they can be expressed to produce the isoenzyme-specific and/or allele-specific peptides for antibody production. Such antibodies may also be used as markers for the purpose of both identifying parental lines and germplasms and monitoring the stacking of genes in new lines, using conventional immunoassays such as, for example, ELISA and western blotting.

10

A further aspect of the present invention utilises the above-mentioned nucleic acid based assay method in the breeding and/or selection of plants which express or do not express particular starch synthase isoenzymes or alternatively, which express a particular starch synthase isoenzyme at a particular level in one or more plant tissues.

15 This aspect clearly extends to the selection of transformed plant material which contains one or more of the isolated nucleic acid molecules of the present invention.

Yet another aspect of the present invention provides for the expression of the nucleic acid molecule of the present invention in a suitable host (e.g. a prokaryote or
20 eukaryote) to produce full length or non-full length recombinant starch synthase gene products.

Hereinafter the term "starch synthase gene product" shall be taken to refer to a recombinant product of a starch synthase gene of the present invention.

25

Preferably, the recombinant starch synthase gene product comprises an amino acid sequence having the catalytic activity of a starch synthase polypeptide or a functional mutant, derivative part, fragment, or analogue thereof.

30 In a particularly preferred embodiment of the invention, the recombinant starch synthase gene product is selected from the following:

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- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;
- 5 (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10; and
- (iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:
- 10 (a) KVGGLGDVVTs;
 (b) GHTVEVILPKY;
 15 (c) HDWSSAPVAWLYKEHY;
 (d) GILNGIDPDIWDPYTD;
 (e) DVPIVGIITRLTAQKG;
 (f) NGQVVLLGSA;
 (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS;
 20 (h) TGGLVDTV;
- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;
- 25 (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;
- 30 (iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at

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least 25% identity to an amino acid sequence selected from the group consisting of:

- (a) KVGGLGDVVTS;
- (b) GHTVEVILPKY;
- 5 (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIIITRLTAQKG;
- (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFPCGLTQLVAMRYGS; and
- 10 (h) TGGLVDTV;
- (i) KTGGLGDVAGA;
- (j) GHRVMVVVPRY;
- (k) NDWHTALLPVYLKAYY;
- (l) GIVNGIDNMEWNPEVD;
- 15 (m) DVPLLGFIGRLDGQKG;
- (n) DVQLVMLGTG;
- (o) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (p) VGG(V/L)RDTV.

20 Accordingly, the present invention clearly extends to homologues, analogues and derivatives of the amino acid sequences set forth herein as SEQ ID NOS: 2, 4, 6, 8 and 10.

In the present context, "homologues" of an amino acid sequence refer to those
 25 polypeptides, enzymes or proteins which have a similar catalytic activity to the amino acid sequences described herein, notwithstanding any amino acid substitutions, additions or deletions thereto. A homologue may be isolated or derived from the same or another plant species as the species from which the polypeptides of the invention are derived.

30

"Analogues" encompass polypeptides of the invention notwithstanding the occurrence

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of any non-naturally occurring amino acid analogues therein.

"Derivatives" include modified peptides in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides or reporter molecules such as radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject peptides are particularly contemplated by the present invention. Additionally, derivatives of an amino acid sequence described herein which comprises fragments or parts of the subject amino acid sequences are within the scope of the invention, as are homopolymers or heteropolymers comprising two or more copies of the subject polypeptides. Procedures for derivatizing peptides are well-known in the art.

Substitutions encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which an amino acid residue contained in a starch synthase gene product is replaced with another naturally-occurring amino acid of similar character, for example Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln or Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a starch synthase gene product described herein is substituted with an amino acid with different properties, such as a naturally-occurring amino acid from a different group (eg. substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Non-conventional amino acids encompassed by the invention include, but are not limited to those listed in Table 2.

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

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Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to the N-terminus, the C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions and of the order of 1-4 amino acid residues.

A homologue, analogue or derivative of a starch synthase gene product as referred to herein may readily be made using peptide synthetic techniques well-known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Techniques for making substituent mutations at pre-determined sites using recombinant DNA technology, for example by M13 mutagenesis, are also well-known. The manipulation of nucleic acid molecules to produce variant peptides, polypeptides or proteins which manifest as substitutions, insertions or deletions are well-known in the art.

15

The starch synthase gene products described herein may be derivatized further by the inclusion or attachment thereto of a protective group which prevents, inhibits or slows proteolytic or cellular degradative processes. Such derivatization may be useful where the half-life of the subject polypeptide is required to be extended, for example to increase the amount of starch produced in the endosperm or alternatively, to increase the amount of protein produced in a bacterial or eukaryotic expression system. Examples of chemical groups suitable for this purpose include, but are not limited to, any of the non-conventional amino acid residues listed in Table 2, in particular a D-stereoisomer or a methylated form of a naturally-occurring amino acid listed in Table 1. Additional chemical groups which are useful for this purpose are selected from the list comprising aryl or heterocyclic N-acyl substituents, polyalkylene oxide moieties, desulphatohirudin muteins, alpha-muteins, alpha-aminophosphonic acids, water-soluble polymer groups such as polyethylene glycol attached to sugar residues using hydrazone or oxime groups, benzodiazepine dione derivatives, glycosyl groups such as beta-glycosylamine or a derivative thereof, isocyanate conjugated to a polyol functional group or polyoxyethylene polyol capped with diisocyanate, amongst others. Similarly, a starch synthase gene product or a homologue, analogue or derivative

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thereof may be cross-linked or fused to itself or to a protease inhibitor peptide, to reduce susceptibility of said molecule to proteolysis.

In a particularly preferred embodiment, the percentage similarity to in any one of SEQ
5 ID NOS: 2, 4, 6, 8 or 10 is at least about 90%, more preferably at least about 95%,
even more preferably at least about 97% and even more preferably at least about
98%, or about 99% or 100%.

In a related embodiment, the present invention provides a "sequencably pure" form of
10 the amino acid sequence described herein. "Sequencably pure" is hereinbefore
described as substantially homogeneous to facilitate amino acid determination.

In a further related embodiment, the present invention provides a "substantially
homogeneous" form of the subject amino acid sequence, wherein the term
15 "substantially homogeneous" is hereinbefore defined as being in a form suitable for
interaction with an immunologically interactive molecule. Preferably, the polypeptide
is at least 20% homogeneous, more preferably at least 50% homogeneous, still more
preferably at least 75% homogeneous and yet still more preferably at least about 95-
100% homogenous, in terms of activity per microgram of total protein in the protein
20 preparation.

To produce the recombinant polypeptide of the present invention, the coding region
of a starch synthase gene described herein or a functional homologue, analogue or
derivative thereof is placed operably in connection with a promoter sequence in the
25 sense orientation, such that a starch synthase gene product is capable of being
expressed under the control of said promoter sequence.

In the present context, the term "in operable connection with" means that expression
of the isolated nucleotide sequence is under the control of the promoter sequence with
30 which it is connected, regardless of the relative physical distance of the sequences
from each other or their relative orientation with respect to each other.

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Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. A promoter is usually, but not necessarily, positioned upstream or 5', of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene.

10

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of a structural gene or other nucleic acid molecule, particularly in a plant cell and more preferably in a wheat plant or other monocotyledonous plant cell, tissue or organ. Preferred promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression and/or to alter the spatial expression and/or temporal expression. For example, regulatory elements which confer copper inducibility may be placed adjacent to a heterologous promoter sequence, thereby conferring copper inducibility on the expression of said molecule.

20

Those skilled in the art will be aware that in order to obtain optimum expression of the starch synthase gene of the present invention, it is necessary to position said gene in an appropriate configuration such that expression is controlled by the promoter sequence. Promoters are generally positioned 5' (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from

30

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which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Examples of promoters suitable for expressing the starch synthase gene of the present invention include viral, fungal, bacterial, animal and plant derived promoters capable of functioning in prokaryotic or eukaryotic cells. Preferred promoters are those capable of regulating the expression of the subject starch synthase genes in plants cells, fungal cells, insect cells, yeast cells, animal cells or bacterial cells, amongst others. Particularly preferred promoters are capable of regulating expression of the subject nucleic acid molecules in monocotyledonous plant cells. The promoter may regulate the expression of the said molecule constitutively, or differentially with respect to the tissue in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or plant pathogens, or metal ions, amongst others.

15

Accordingly, strong constitutive promoters are particularly preferred for the purposes of the present invention.

Examples of preferred promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, *lac* operator-promoter, *tac* promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, CaMV 35S promoter, SCSV promoter, SCBV promoter and the like.

Particularly preferred promoters operable in plant cells include, for example the CaMV 35S promoter, and the SCBV promoter. Those skilled in the art will readily be aware of additional promoter sequences other than those specifically described.

In a particularly preferred embodiment, the promoter may be derived from a genomic starch synthase gene. Preferably, the promoter sequence comprises nucleotide sequences that are linked *in vivo* to nucleotide sequences set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11-16, 37, or 38. By "linked *in vivo*" means that the promoter is present in its native state in the genome of a wheat plant where it controls expression

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of the starch synthase gene of the present invention.

Conveniently, genetic constructs are employed to facilitate expression of a starch synthase genetic sequence of the present invention or a functional derivative, part, 5 homologue, or analogue thereof. To produce a genetic construct, the starch synthase gene of the invention is inserted into a suitable vector or episome molecule, such as a bacteriophage vector, viral vector or a plasmid, cosmid or artificial chromosome vector which is capable of being maintained and/or replicated and/or expressed in the host cell, tissue or organ into which it is subsequently introduced. The said genetic 10 construct comprises the subject nucleic acid molecule placed operably under the control of a promoter sequence and optionally, a terminator sequence.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA 15 sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in bacteria, yeasts, animal cells and plant cells are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

20 Examples of terminators particularly suitable for use in expressing the nucleic acid molecule of the present invention in plant cells include the nopaline synthase (NOS) gene terminator of *Agrobacterium tumefaciens*, the terminator of the Cauliflower mosaic virus (CaMV) 35S gene, and the *zein* gene terminator from *Zea mays*.

25 Genetic constructs will generally further comprise one or more origins of replication and/or selectable marker gene sequences.

The origin of replication can be functional in a bacterial cell and comprise, for example, the pUC or the ColE1 origin. Alternatively, the origin of replication is operable in a 30 eukaryotic cell, tissue and more preferably comprises the 2 micron (2 μ m) origin of replication or the SV40 origin of replication.

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As used herein, the term "selectable marker gene" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a genetic construct of the invention or a derivative thereof.

5

Suitable selectable marker genes contemplated herein include the ampicillin-resistance gene (Amp^r), tetracycline-resistance gene (Tc^r), bacterial kanamycin-resistance gene (Kan^r), is the zeocin resistance gene (Zeocin is a drug of bleomycin family which is trademark of InVitrogen Corporation), the *AURI-C* gene which confers resistance to the
 10 antibiotic aureobasidin A, phosphinothricin-resistance gene, neomycin phosphotransferase gene (*nptII*), hygromycin-resistance gene, β -glucuronidase (GUS) gene, chloramphenicol acetyltransferase (CAT) gene, green fluorescent protein-encoding gene or the luciferase gene, amongst others. Those skilled in the art will be aware of other selectable marker genes useful in the performance of the present
 15 invention and the subject invention is not limited by the nature of the selectable marker gene.

Usually, an origin of replication or a selectable marker gene suitable for use in bacteria is physically-separated from those genetic sequences contained in the genetic
 20 construct which are intended to be expressed or transferred to a eukaryotic cell, or integrated into the genome of a eukaryotic cell.

Standard methods can be used to introduce genetic constructs into a cell, tissue or organ for the purposes of modulating gene expression. Particularly preferred methods
 25 suited to the introduction of synthetic genes and genetic constructs comprising same to eukaryotic cells include liposome-mediated transfection or transformation, transformation of cells with attenuated virus particles or bacterial cells and standard procedures for the transformation of plant and animal cells, tissues, organs or organisms. Any standard means may be used for their introduction including cell
 30 mating, transformation or transfection procedures known to those skilled in the art or described by Ausubel *et al.* (1992).

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In a further embodiment of the present invention, the starch synthase genes of the present invention and genetic constructs comprising same are adapted for integration into the genome of a cell in which it is expressed. Those skilled in the art will be aware that, in order to achieve integration of a genetic sequence or genetic construct into the genome of a host cell, certain additional genetic sequences may be required. In the case of plants, left and right border sequences from the T-DNA of the *Agrobacterium tumefaciens* Ti plasmid will generally be required.

The invention further contemplates increased starch and/or modified starch composition in transgenic plants expressing the nucleic acid molecule of the invention in the sense orientation such that the activity of one or more starch synthase isoenzymes is increased therein. By increasing the level of one or more starch synthase isoenzymes, the deposition of starch in the amyloplast or chloroplast is increased and/or a modified starch granule structure is produced and/or starch composition is modified and/or the amylose/amylopectin ratio is altered in the plant.

Wherein it is desired to increase the synthesis of a particular starch synthase isoenzyme in a plant cell, the coding region of a starch synthase gene is placed operably behind a promoter, in the sense orientation, such that said starch synthase is expressed under the control of said promoter sequence. In a preferred embodiment, the starch synthase genetic sequence is a starch synthase genomic sequence, cDNA molecule or protein-coding sequence.

Wherein it is desirable to reduce the level of a particular starch synthase isoenzyme in a plant cell, the nucleic acid molecule of the present invention can be expressed in the antisense orientation, as an antisense molecule or a ribozyme molecule, under the control of a suitable promoter.

Alternatively, the nucleic acid molecule of the present invention may also be expressed in the sense orientation, in the form of a co-suppression molecule, to reduce the level of a particular starch synthase isoenzyme in a plant cell. As will be known to those skilled in the art, co-suppression molecules that comprise inverted repeat sequences

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of a target nucleic acid molecule provide optimum efficiency at reducing expression of said target nucleic acid molecule and, as a consequence, the present invention clearly contemplates the use of inverted repeat sequences of any one or more of the starch synthase genetic sequences exemplified herein, or inverted repeat sequences of a
5 homologue, analogue or derivative of said starch synthase genetic sequences, to reduce the level of a starch synthase isoenzyme in a plant.

The expression of an antisense, ribozyme or co-suppression molecule comprising a starch synthase gene in a cell such as a plant cell, fungal cell, insect cell, animal cell,
10 yeast cell or bacterial cell, may also increase the availability of carbon as a precursor for a secondary metabolite other than starch (e.g. sucrose or cellulose). By targeting the endogenous starch synthase gene, expression is diminished, reduced or otherwise lowered to a level that results in reduced deposition of starch in the amyloplast or chloroplast and/or leads to modified starch granule structure and/or composition
15 and/or altered amylose/amylopectin ratio.

Accordingly, a further aspect of the present invention provides a method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing therein a sense molecule, antisense molecule, ribozyme
20 molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified. This aspect of the invention clearly extends to the
25 introduction of the sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule to isolated plant cells, tissues or organs or organelles by cell fusion or transgenic means and the regeneration of intact plants therefrom.

30 Co-suppression is the reduction in expression of an endogenous gene that occurs when one or more copies of said gene, or one or more copies of a substantially similar

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gene are introduced into the cell, preferably in the form of an inverted repeat structure.

The present inventors have discovered that the genetic sequences disclosed herein are capable of being used to modify the level of starch when expressed, particularly when expressed in plants cells. Accordingly, the present invention clearly extends to the modification of starch biosynthesis in plants, in particular wheat or barley plants or a progenitor plant species, or a relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others.

10

In particular, the present invention contemplates decreased starch production and/or modified starch composition in transgenic plants expressing the nucleic acid molecule of the invention in the antisense orientation or alternatively, expressing a ribozyme or co-suppression molecule comprising the nucleic acid sequence of the invention such that the activity of one or more starch synthase isoenzymes is decreased therein.

15

In the context of the present invention, an antisense molecule is an RNA molecule which is transcribed from the complementary strand of a nuclear gene to that which is normally transcribed to produce a "sense" mRNA molecule capable of being translated into a starch synthase polypeptide. The antisense molecule is therefore complementary to the mRNA transcribed from a sense starch synthase gene or a part thereof. Although not limiting the mode of action of the antisense molecules of the present invention to any specific mechanism, the antisense RNA molecule possesses the capacity to form a double-stranded mRNA by base pairing with the sense mRNA, which may prevent translation of the sense mRNA and subsequent synthesis of a polypeptide gene product.

25

Ribozymes are synthetic RNA molecules which comprise a hybridising region complementary to two regions, each of at least 5 contiguous nucleotide bases in the target sense mRNA. In addition, ribozymes possess highly specific endoribonuclease

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activity, which autocatalytically cleaves the target sense mRNA. A complete description of the function of ribozymes is presented by Haseloff and Gerlach (1988) and contained in International Patent Application No. WO89/05852.

- 5 The present invention extends to ribozyme which target a sense mRNA encoding a native starch synthase gene product, thereby hybridising to said sense mRNA and cleaving it, such that it is no longer capable of being translated to synthesise a functional polypeptide product.
- 10 According to this embodiment, the present invention provides a ribozyme or antisense molecule comprising at least 5 contiguous nucleotide bases derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto or a homologue, analogue or derivative thereof, wherein said antisense or ribozyme molecule is able to form a hydrogen-bonded complex with a sense mRNA
- 15 encoding a starch synthase gene product to reduce translation thereof.

In a preferred embodiment, the antisense or ribozyme molecule comprises at least 10 to 20 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto or a homologue, analogue

20 or derivative thereof. Although the preferred antisense and/or ribozyme molecules hybridise to at least about 10 to 20 nucleotides of the target molecule, the present invention extends to molecules capable of hybridising to at least about 50-100 nucleotide bases in length, or a molecule capable of hybridising to a full-length or substantially full-length mRNA encoded by a starch synthase gene.

25

Those skilled in the art will be aware of the necessary conditions, if any, for selecting or preparing the antisense or ribozyme molecules of the invention.

It is understood in the art that certain modifications, including nucleotide substitutions

30 amongst others, may be made to the antisense and/or ribozyme molecules of the present invention, without destroying the efficacy of said molecules in inhibiting the expression of a starch synthase gene. It is therefore within the scope of the present

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invention to include any nucleotide sequence variants, homologues, analogues, or fragments of the said gene encoding same, the only requirement being that said nucleotide sequence variant, when transcribed, produces an antisense and/or ribozyme molecule which is capable of hybridising to a sense mRNA molecule which
5 encodes a starch synthase gene product.

Gene targeting is the replacement of an endogenous gene sequence within a cell by a related DNA sequence to which it hybridises, thereby altering the form and/or function of the endogenous gene and the subsequent phenotype of the cell. According
10 to this embodiment, at least a part of the DNA sequence defined by any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38 may be introduced into target cells containing an endogenous gene that encodes a particular starch synthase isoenzyme, thereby replacing said endogenous gene. According to this embodiment, the polypeptide product of the gene targeting molecule generally encodes a starch synthase
15 isoenzyme that possesses different catalytic activity to the polypeptide product of the endogenous gene, producing in turn modified starch content and/or composition in the target cell.

The present invention extends to genetic constructs designed to facilitate expression
20 of a sense molecule, an antisense molecule, ribozyme molecule, co-suppression molecule, or gene targeting molecule of the present invention. The requirements for expressing such molecules are similar to those for expressing a recombinant polypeptide as described *supra*.

25 The present invention further extends to the production and use of starches and proteins produced using the novel genes described herein. Modified starches produced by plants which have been selected using marker-assisted selection, or alternatively, produced by transgenic plants carrying the introduced starch synthase genes, are particularly suitable for use in food products, such as, for example, flour
30 and flour-based products, in particular those products selected from the group consisting of: flour-based sauce; leavened bread; unleavened bread; pasta, noodle; cereal; snack food; cake; and pastry. Modified proteins are also suitable for use in non-

food products, such as, for example, those non-food products selected from the group consisting of: films; coatings; adhesives; building materials; and packaging materials.

Additionally, starch hydrolysates or undegraded starches are both useful in industry and, as a consequence, the present invention is useful in applications relating to the use of both starch hydrolysates and undegraded starches. By "starch hydrolysates" is meant the glucose and glucan components that are obtainable by the enzymatic or chemical degradation of starch in chemical modifications and processes, such as fermentation.

10

Starch produced by plants expressing the sense, antisense, co-suppression, gene-targeting or ribozyme molecules of the present invention may exhibit modified viscosities and/or gelling properties of its gels when compared to starch derived from wild-type plants. Native starches produced by the performance of the inventive method are useful as an additive in the following: (i) foodstuffs, for the purpose of increasing the viscosity or gelling properties of food; (ii) in non-foodstuffs, such as an adjuvant or additive in the paper and cardboard industries, for retention or as a size filler, or as a solidifying substance or for dehydration, or film coating, amongst others; (iii) in the adhesive industry as pure starch glue, as an additive to synthetic resins and polymer dispersions, or as an extenders for synthetic adhesives; (iv) in the textile and textile care industries to strengthen woven products and reduce burring or to thicken dye pastes; (v) in the building industry, such as a binding agent in the production of gypsum plaster boards, or for the deceleration of the sizing process; (vi) in ground stabilization or for the temporary protection of ground particles against water in artificial earth shifting; (vii) as a wetting agent in plant protectants and fertilizers; (viii) as a binding agent in drugs, pharmaceuticals and medicated foodstuff such as vitamins, etc; (ix) as an additive in coal and briquettes; (xi) as a flocculent in the processing of coal ore and slurries; (xii) as a binding agent in casting processes to increase flow resistance and improve binding strength; and (xiii) to improve the technical and optical quality of rubber and plastic products. Additional applications are not excluded.

30

A further aspect of the present invention provides an isolated promoter that is operable

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in the endosperm of a monocotyledonous plant cell, tissue or organ, and preferably in the endosperm of a monocotyledonous plant cell, tissue or organ. According to this embodiment, it is preferred that the promoter is derived from a starch synthase gene of the present invention, such as a promoter that is linked *in vivo* to any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto.

In a particularly preferred embodiment, the promoter comprises a nucleotide sequence derivable from the 5'-upstream region of SEQ ID NO: 11 or SEQ ID NO: 37 or SEQ ID NO: 38, or a complementary nucleotide sequence thereto, and more preferably comprises nucleotides 1 to about 287 of SEQ ID NO: 11, or nucleotides 1 to about 1416 of SEQ ID NO: 37, or nucleotides 1 to about 973 of SEQ ID NO: 38, or a complementary nucleotide sequence thereto. The present invention clearly extends to promoter sequences that comprise further nucleotide sequences in the region upstream of the stated nucleotide sequence that are linked *in vivo* to said nucleotide sequence in the wheat genome.

In a related embodiment, the promoter sequence of the present invention will further comprise an exon sequence derived from a starch synthase gene, such as, for example, an intron I sequence described herein, or a complementary nucleotide sequence thereto. Those skilled in the art will be aware that the inclusion of such nucleotide sequences may increase the expression of a heterologous structural gene, the expression of which is controlled thereby. Preferred intron I sequences include, for example, nucleotide sequences in the region of about position 1744 to about 1847 of SEQ ID NO: 37, and/or about position 1100 to about position 2056 of SEQ ID NO: 38. Additional sequences comprising intron/exon junction boundary sequences which are readily determined by those skilled in the art are not excluded.

The present invention further extends to the expression of any structural gene operably under the control of the starch synthase promoter sequence exemplified herein or a functional homologue, analogue or derivative of said promoter sequence.

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As with other embodiments described herein for expression in cells, a genetic construct may be employed to effect said expression and the present invention clearly extends to said genetic constructs.

- 5 The polypeptide encoded by the structural gene component may be a reporter molecule which is encoded by a gene such as the bacterial β -glucuronidase gene or chloramphenicol acetyltransferase gene or alternatively, the firefly luciferase gene. Alternatively, wherein it is desirable to alter carbon partitioning within the endosperm, the polypeptide may be an enzyme of the starch sucrose biosynthetic pathways.
- 10 Preferably, the promoter sequence is used to regulate the expression of one or more of the starch synthase genes of the present invention or a sense, antisense, ribozyme, co-suppression or gene-targetting molecule comprising or derived from same.

Recombinant DNA molecules carrying the aforesaid nucleic acid molecule of the present invention or a sense, antisense, ribozyme, gene-targetting or co-suppression molecule and/or genetic construct comprising same, may be introduced into plant tissue, thereby producing a "transgenic plant", by various techniques known to those skilled in the art. The technique used for a given plant species or specific type of plant tissue depends on the known successful techniques. Means for introducing recombinant DNA into plant tissue include, but are not limited to, transformation (Paszkowski *et al.*, 1984), electroporation (Fromm *et al.*, 1985), or microinjection of the DNA (Crossway *et al.*, 1986), or T-DNA-mediated transfer from *Agrobacterium* to the plant tissue. Representative T-DNA vector systems are described in the following references: *Ali et al.* (1985); Herrera-Estrella *et al.* (1983a, b); Herrera-Estrella *et al.* (1985). Once introduced into the plant tissue, the expression of the introduced gene may be assayed in a transient expression system, or it may be determined after selection for stable integration within the plant genome. Techniques are known for the *in vitro* culture of plant tissue, and in a number of cases, for regeneration into whole plants. Procedures for transferring the introduced gene from the originally transformed plant into commercially useful cultivars are known to those skilled in the art.

In general, plants are regenerated from transformed plant cells or tissues or organs on

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hormone-containing media and the regenerated plants may take a variety of forms, such as chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., a transformed root stock grafted to an untransformed scion in citrus species). Transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plants may be selfed to give homozygous second generation (or T2) transformed plants, and the T2 plants further propagated through classical breeding techniques.

10

Accordingly, a still further aspect of the present invention contemplates a transgenic plant comprising an introduced sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto or a genetic construct comprising same. The present invention further extends to those plant parts, propagules and progeny of said transgenic plant or derived therefrom, the only requirement being that said propagules and progeny also carry the introduced nucleic acid molecule(s).

20

The present invention is further described by reference to the following non-limiting examples.

EXAMPLE 1

Plant material

25 Genetic stocks of hexaploid bread wheat *Triticum aestivum* cv. Chinese Spring with various chromosome additions and deletions were kindly supplied by Dr E. Lagudah (CSIRO Plant Industry, Canberra) and derived from stocks described in Sears and Miller (1985). The hexaploid (*Triticum aestivum*) wheats cv Gabo and cv Wyuna were grown in controlled growth cabinet conditions (18°C day and 13°C night, with a photoperiod of 16 h). Wheat leaves and florets prior to anthesis, and endosperm were collected over the grain filling period, immediately frozen in liquid nitrogen and stored

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at -80°C until required.

EXAMPLE 2

Gel Electrophoresis, Antibodies and Immunoblotting

- 5 Monoclonal antibodies against the Sgp-1 proteins, and their use in the immunoblotting of SDS-PAGE gels have been described previously (Rahman *et al.*, 1995).

EXAMPLE 3

Preparation of total RNA from wheat

- 10 Total RNA was isolated from the leaf, floret and endosperm tissues of wheat essentially as described by Higgins *et al.* (1976) or Rahman *et al.* (1998). RNA was quantified by UV absorption and by separation in 1.4% (w/v) agarose-formaldehyde gels which were then visualised under UV light after staining with ethidium bromide.

EXAMPLE 4

Construction and screening of cDNA libraries

- 15 A first cDNA library, an expression cDNA library of wheat endosperm, was constructed from mRNA isolated from wheat cv Chinese Spring. RNA from 5, 7, 9, 11 and 13 days after anthesis was pooled and random primers were used for the first strand of cDNA synthesis. Monoclonal antibodies against 100 -105 kDa proteins in wheat starch granules (Rahman *et al.*, 1995) were used for immunoscreening of the expression cDNA library.

- A second cDNA library was constructed from the endosperm mRNA of the hexaploid
25 *Triticum aestivum* cultivar Wyuna, 8 - 12 days after anthesis, as described by Rahman *et al.* (1997). This library was screened with a 85-bp cDNA fragment, wSSIIP1, which was obtained by immunoscreening of the expression cDNA library as described above. The wSSIIP1 probe corresponded to nucleotide positions 988 to 1072 of wSSIIB (SEQ ID NO:1) at the hybridisation conditions as described earlier (Rahman *et al.*, 1998).

30

A third cDNA library was constructed from RNA from the endosperm of the hexaploid

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Triticum aestivum cultivar Rosella as described by Rahman *et al.* (1997). This library was screened with a 347-bp cDNA fragment, wSSIIIp1 for the first screening, and a 478-bp cDNA fragment wSSIIIp3 for the second screening using the hybridisation conditions described herein.

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EXAMPLE 5

Construction and screening of *Triticum tauschii* genomic library

The genomic library used in this study, prepared from *Triticum tauschii*, var strangulata, (Accession Number CPI 110799), has been described in Rahman *et al.*,
10 (1997). Of all the accessions of *T. tauschii* surveyed, DNA marker analysis suggests that the genome of CPI 110799 is the most closely related to the D genome of hexaploid wheat (Lagudah *et al.*, 1991).

Hybridisations were carried out in 25% formamide, 6 x SSC, 0.1% SDS at 42°C for 16
15 hours, then filters were washed 3 times using 2 x SSC containing 0.1% SDS at 65°C for 1 hour per wash.

For the isolation of a genomic wSSII clone, the probe comprised the PCR-derived DNA fragment wSSIIp2 and positive-hybridising plaques were digested using the restriction
20 enzyme *Bam*HI, separated on a 1% agarose gel, transferred to nitrocellulose membrane and hybridised to probe wSSIIp4 comprising nucleotides 1 to 367 of the wSSIIA cDNA clone, using the conditions described by Rahman *et al.* (1997).

For the isolation of a genomic wSSIII clone, plaques hybridising to the PCR-derived
25 DNA fragment wSSIIIp1 from clone wSSIII.B3 (i.e. nucleotides 3620 to 3966 of SEQ ID NO:7) were selected and re-screened until plaque-purified.

EXAMPLE 6

DNA sequencing and analysis

30 DNA sequencing was performed using the automated ABI system with dye terminators as described by the manufacturers. DNA sequences were analysed using the GCG

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suite of programs (Devereaux *et al.*, 1984).

EXAMPLE 7

DNA and RNA analysis

5 DNA was isolated and analysed as previously described (Maniatis *et al.*, 1982; Rahman *et al.*, 1998). Approximately 20 μ g of DNA was digested with restriction enzymes *Bam*HI, *Dra*I and *Eco*RI, separated on a 1% agarose gel and transferred to reinforced nitrocellulose membranes (BioRad) and hybridised with 32 P-labelled DNA probe, either wSSIIIp1, corresponding to nucleotides 3620 to 3966 of the wheat SSIII
10 gene, or alternatively, with the entire wSSII cDNA clone. DNA fragment probes were labelled with the Rapid Multiprime DNA Probe Labelling Kit (Promega).

The hybridisation and wash conditions were performed as described in Rahman *et al.* (1997). For RNA analysis, 10 μ g of total RNA was separated in a 1.4% agarose-
15 formaldehyde gel and transferred to a Hybond N+ membrane (Amersham), and hybridised with cDNA probe at 42°C as previously described by Khandjian *et al.*, (1987) or Rahman *et al.*, (1998). After washing for 30 minutes at 65°C with 2x SSC, 0.1% SDS; followed by three washes of 40 minutes at 65°C with 0.2x SSC, 1% SDS, the membranes were visualised by overnight exposure at -80°C with Kodak MR X-ray
20 film.

EXAMPLE 8

Expression of wheat Sgp-1 polypeptides in the wheat endosperm

The development and use of monoclonal antibodies to the Sgp-1 proteins has been
25 described previously (Rahman *et al.*, 1995). These antibodies were used by the present inventors to characterise the expression and localisation of the Sgp-1 proteins.

The proteins found in the matrix of the wheat starch granule are shown in Figure 1, lane 1. The remaining lanes show an immunoblot of proteins from the soluble phase
30 (Figure 1; lanes 2-4) and the starch granule (Figure 1; lanes 5-7), respectively, following SDS-PAGE. In addition to cross-reactivity with the 100-105 kDa proteins, a

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weak cross-reaction with a 50 kDa protein in both the granule and the soluble fractions were observed (Figure 1). The Sgp-1 polypeptides are present in the starch granule throughout endosperm development (Figure 1; lanes 5-7, also see Rahman *et al.*, 1995). However, as the endosperm matures, there is a reduction in the amount of Sgp-1 protein found in the soluble fraction. Lane 4 shows that by 25 days after anthesis, the level of these proteins in the soluble fraction is substantially reduced. This observation is consistent with previous results from Rahman *et al.*, (1995), who suggested that the Sgp-1 proteins were exclusively granule bound based on studies of granules from endosperm in mid-late stages endosperm development, however, these results suggest that the partitioning of these proteins between the granule and the soluble phase changes during development.

EXAMPLE 9

Isolation of cDNA clones encoding wheat starch synthase II (wSSII) proteins

Monoclonal antibodies against Sgp-1 polypeptides (Rahman *et al.*, 1995) were used to probe the expression library described in Example 4 (i.e. the first cDNA library). Three immunoreactive plaques were identified and sequenced. One clone, designated wSSIIp1, contained an 85-bp cDNA insert with homology to maize SSIIa (Harn *et al.*, 1998).

20

DNA from the wSSIIp1 clone was used as a probe in the hybridisation screening of the second cDNA library, prepared from *Triticum aestivum* cultivar Wyuna endosperm RNA as described in Example 4. Ten hybridising cDNA clones were selected and sequenced. On the basis of the DNA sequences obtained, the 10 cDNA clones can be classified into three groups. Group 1 contains 7 cDNA clones, group 2 contains 2 cDNA clones and group 3 contains 1 cDNA clone.

The longest clone from group 1 (designated wSSIIb) is 2939 bp in length (SEQ ID NO:1) and encodes a 798 -amino acid polypeptide in the region from nucleotide position 176 to nucleotide position 2569 (SEQ ID NO:2).

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The longest clone from group 2 (designated wSSIIA) is 2842 bp in length (SEQ ID NO:3) and encodes a 799 -amino acid polypeptide in the region from nucleotide position 89 to nucleotide position 2485 (SEQ ID NO:4).

- 5 The cDNA from group 3 is a partial cDNA clone (designated wSSIID), which is 2107 bp in length (SEQ ID NO:5) and encodes a 597 -amino acid polypeptide in the region from nucleotide position 1 to nucleotide position 1791 (SEQ ID NO:6). The encoded polypeptide is approximately a 200 amino acid residues shorter than that of polypeptides encoded by longest clones of group 1 or 2 clones, respectively (Figure 10 2).

Comparison of the three cDNA clones, wSSIIB, wSSIIA and wSSIID shows that they share 95.7% to 96.6% identity at the amino acid level, with variation at 44 amino acid positions between the three sequences (Figure 3). Of the 44 amino acid changes between these sequences, 31 changes occur in the N-terminal region (residues 1 to 300), 10 changes occur in the central region (residues 301 to 729) and 3 changes occur in the C-terminal region (residues 730 to 799). The wSSIIA polypeptide (799 amino acid residues) and wSSIIB polypeptide (798 amino acid residues) sequences differ in length by a single amino acid residue, due to the deletion of Asp-69 from the 20 wSSIIB polypeptide sequence.

A comparison of the nucleotide sequences of the wSSIA, wSSIIB and wSSIID cDNA clones with the nucleotide sequence of the wSSIIP1 cDNA obtained by immunoscreening confirms that the wSSIIP1 sequence is found in each cDNA (Figure 25 3). The peptide encoded by the wSSIIP1 cDNA clone corresponds to amino acid residues in the region from residue 272 to residue 298 of the wSSIIA polypeptide, and to amino acid residues in the region from residue 271 to residue 297 of the wSSIIB polypeptide (see Figure 3). Thus, the peptide epitope encoded by wSSIIP1 that reacts with the anti-Sgp-1 monoclonal antibodies can therefore be localised to this region of 30 the wSSIIA and wSSIIB polypeptides and to the corresponding region of the wSSIID polypeptide.

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Notwithstanding that a region having about 63% amino acid sequence identity to the peptide epitope encoded by clone wSSIIP1 is found in the maize SSIIa polypeptide (Figure 3), the degree of amino acid conservation between maize and wheat sequences in this region of the polypeptide is insufficient for immunological cross-reactivity to occur between these species using the monoclonal antibodies to the wheat Sgp-1 proteins described by Rahman *et al.* (1995). Additionally, this peptide epitope is not found in granule-bound starch synthases, SSI, or SSIII (data not shown).

The wSSIIB cDNA (SEQ ID NO:1) encodes an amino acid sequence comprising the peptide motif AAGKKDAGID (SEQ ID NO: 18) between residues 60 and 69 of SEQ ID NO:2 (Figure 3) which, with the exception of the second residue, is identical to the N-terminal of the 100 kDa (A^T_LGKKDAGID: SEQ ID NOS:19 and 20) protein (Sgp-B1) from the wheat starch granule (note that the sequence given in Rahman *et al.*, 1995 (A^T_LGKKDAL: SEQ ID NOS: 21 and 22) has been revised following further amino acid sequence analysis).

The wSSIa cDNA clone (SEQ ID NO:3) encodes an amino acid sequence comprising the peptide motif AAGKKDARVDDDA (SEQ ID NO: 23) at residues 60 to 73 of SEQ ID NO:4, which is about 66% identical to the N-terminal amino acid sequence (i.e. ALGKKDAGIVDGA: SEQ ID NO: 24) of the 104 kDa and 105 kDa starch granule proteins, Sgp-D1 and Sgp-A1 respectively, as determined by sequence analysis of isolated protein (Rahman *et al.*, 1995).

Furthermore, Takaoka *et al.* (1997) reported the amino acid sequences of 3 polypeptides obtained from sequencing starch granule proteins derived from the Sgp-1 proteins. Peptide 3 described by Takaoka *et al.* (1997) corresponds to amino acid residues 378 to 387 of the amino acid sequence of the wSSIa cDNA (SEQ ID NO:4; Figure 3). Peptides 1 and 2 described by Takaoka *et al.* (1997) could not be detected in the amino acid sequences of the wSSII cDNA clones of the present invention, however peptide 1 of Takaoka *et al.* (1997) can be found in the amino acid sequences of SSI from maize, rice, wheat and potato (data not shown).

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Denyer *et al.* (1995) demonstrated that the Sgp-1 proteins possess starch synthase activity and, as a consequence, the wSSIIB, wSSIA and wSSIID cDNA clones encode starch synthase enzymes that are differentially expressed in a developmentally-regulated manner in both the soluble and granule-bound fractions of the endosperm (Figure 1). Based on the nomenclature suggested by Harn *et al.* (1998), it is appropriate to describe the Sgp-1 proteins as "starch synthases" rather than "granule-bound starch synthases".

EXAMPLE 10

10 **Analysis of wheat starch synthase II mRNA expression**

The mRNA for wheat starch synthase II could be detected in leaves, pre-anthesis florets and endosperm of wheat when total RNAs isolated from these tissue were probed with a PCR probe, wSSIIP2, corresponding to nucleotide positions 1435 to 1835 bp of wSSIIB-cDNA (SEQ ID NO:1; Figure 4). Unlike wSSI, which could not be detected in wheat leaves derived from plants grown under the same conditions, wSSII genes are highly-expressed in the leaves (Figure 4, lane 1), and expressed at an intermediate level in pre-anthesis florets (Figure 4, lane 2), and at much lower levels in developing wheat endosperm cells (Figure 4, lanes 3-11). In contrast, the maize SSIIa is expressed predominantly in the endosperm, whilst the maize SSIIb is detected mainly in the leaf, albeit at low levels (Harn *et al.*, 1998).

The wSSII mRNA was detectable in the endosperm 6 days after anthesis and mRNA levels increase between 8 and 18 days post-anthesis, after which time levels of mRNA decline.

Southern blotting experiments in wheat demonstrated that the wSSIIP2 probe used detected only a single copy of the SSII gene in each genome (data not shown). Thus, it is unlikely that this probe cross-hybridised with mRNAs encoded by genes other than wSSII.

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EXAMPLE 11

Chromosomal localization of the wheat wSSII genes.

I. Amplification of specific cDNA regions of wheat starch synthase II using PCR

Two PCR products, wSSIIp2 and wSSIIp3 were amplified from the cDNA clone wSSIIb
5 and used for the northern hybridisation and Southern hybridisation, respectively.

The primers sslIa (5' TGTGAGGTTCATGGCACGTTC 3': SEQ ID NO: 25) and sslIb
(5' AGTCGTTCTGCCGTATGATGTCG 3': SEQ ID NO: 26) were used to amplify the
cDNA fragment wSSIIp2 (i.e. nucleotide positions 1435 to 1835 of SEQ ID NO:1).

10

The primers sslIc (5' CCAAGTACCAGTGGTGAACGC 3': SEQ ID NO: 27) and sslId
(5' CGGTGGGATCCAACGGCCC 3': SEQ ID NO: 28) were used to amplify the cDNA
fragment wSSIIp3 (i.e. nucleotide positions 2556 to 2921 of SEQ ID NO:1).

15 The amplification reactions were performed using a FTS-1 thermal sequencer (Corbett,
Australia) for 1 cycle of 95°C for 2 minutes; 35 cycles of 95°C for 30 seconds, 60°C for
1 minutes, 72°C for 2 minutes and 1 cycle of 25°C for 1 minute.

II. PCR and nucleotide sequence analysis of 3' sequences of wheat SSII genes

20 Genomic DNA was extracted from wild-type Chinese Spring wheat, and from three
nullisomic-tetrasomic lines of chromosome 7 of Chinese Spring wheat, and from
Triticum tauschii (var strangulata, accession number CPI 100799), and used as a
template for the amplification and nucleotide sequence analysis of wheat SSII genes.

25 RFLP analysis of *Bam*HI and *Eco*RI restricted DNA from each wheat or *T. Tauschii* line
was carried out using the wSSIIp3 fragment as a probe. Three hybridising bands were
obtained which could be assigned to chromosomes 7A, 7B and 7D, respectively (data
not shown). This analysis indicates that there is a single copy of the wSSII gene in
each genome in hexaploid wheat, consistent with the findings of Yamamori and Endo
30 (1996) who located the SGP-A1, B1 and D1 proteins to the short arm of chromosome
7.

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PCR analysis was used to assign each of the cDNA clones to the individual wheat genomes. A single 365 bp PCR fragment was obtained from nullisomic-tetrasomic genomic DNA of Chinese Spring when primers *sslIc* and *sslId* were used for the PCR amplification (Figure 5, right panel). This PCR product is obtained only from lines
5 bearing the B genome. The fragment was cloned and sequenced and shown to be identical to a 365 bp region of the *wSSIIB* cDNA. An identical fragment is obtained by PCR amplification of the *wSSIIB* cDNA clone, but not by amplification of the *wSSIIA* or *wSSIID* clones, supporting the conclusion that the *wSSIIB* cDNA is the product of a gene located on chromosome 7 of the B genome of hexaploid wheat.

10

Two PCR products were also amplified from nullisomic-tetrasomic genomic DNA of Chinese Spring using the primers *sslIc* and *sslIe* (Figure 5, left panel). One PCR fragment, approximately 350 bp is only amplified when the A genome is present, and a second 322 bp product is only amplified when the D-genome is present. The 350 and
15 322 bp PCR products were also cloned and sequenced and shown to be identical to the *wSSIIA* and *wSSIID* cDNAs, respectively, supporting the conclusion that the *wSSIIA* and *wSSIID* cDNAs are the products of genes located on chromosomes 7A and 7D, respectively.

20

EXAMPLE 12

Isolation of genomic *wSSII* clones

Screening of a genomic library from the D-genome donor of wheat, *T. tauschii*, was performed as described in Example 5, using the PCR-derived DNA fragment *wSSIIP2* as a hybridisation probe. A positive-hybridising clone, designated *wSSII-8*, and
25 comprising a putative *T. tauschii* homologue of the *wSSII* gene, was isolated.

Positive-hybridising plaques were digested using the restriction enzyme *Bam*HI, separated on a 1% agarose gel, transferred to nitrocellulose membrane and hybridised to probe *wSSIIP4* comprising nucleotides 1 to 367 of the *wSSIIA* cDNA clone, using
30 the conditions described by Rahman *et al.* (1997). Clone *wSSII-8* also hybridises strongly to the *wSSIIP4* probe, confirming its identity as a genomic *wSSII* gene.

The complete nucleotide sequence of the wSSII gene was determined and is presented herein as SEQ ID NO: 37. The structural features of this gene are present in Table 3. A schematic representation of the intron/exon organisation of this gene is also presented in Figure 6.

5

TABLE 3**Structural features of the wheat starch synthase II genomic gene**

	Nucleotide Position in SEQ ID NO: 37	Feature	Length (bases)
10	1- 1416	5'-untranscribed region and promoter sequence	1416
	1417 - 1743	exon 1	327
	1480-1482	translation start codon (ATG)	3
	1744 - 1847	intron 1	104
	1848 - 2553	exon 2	706
15	2554 - 2641	intron 2	88
	2642 - 2706	exon 3	65
	2707 - 3606	intron 3	900
	3607 - 3684	exon 4	78
	3685 - 3773	intron 4	89
20	3774 - 3884	exon 5	111
	3885 - 3981	intron 5	97
	3982 - 4026	exon 6	45
	4027 - 4406	intron 6	380
	4407 - 4580	exon 7	174
25	4581 - 7296	intron 7	2716
	7297 - 8547	exon 8	1251
	8251 - 8253	translation stop codon (TGA)	3
	8548 -9024	3'-untranscribed region	477

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EXAMPLE 13

Cloning of specific cDNA regions of wheat starch synthase III using RT-PCR
PCR primers were used to amplify sequences of starch synthase III from wheat endosperm cDNA. The design of PCR primers was based on the sequences of starch
5 synthase III from potato and the *du1* starch synthase III gene of maize.

First-strand cDNAs were synthesised from 1 μ g of total RNA (derived from endosperm of the cultivar Rosella, 12 days after anthesis) as described by Maniatis *et al.* (1982), and then used as templates to amplify two specific cDNA regions, wSSIIIp1 and
10 wSSIIIp2, of wheat starch synthase III by PCR.

The primers used to obtain the cDNA clone wSSIIIp1 were as follows:
Primer wSS3pa (5' GGAGGTCTTGGTGATGTTGT 3': SEQ ID NO: 29); and
Primer wSS3pb (5' CTTGACCAATCATGGCAATG 3': SEQ ID NO: 30).

15

The primers used to obtain the cDNA clone wSSIIIp2 were as follows:
Primer wSS3pc (5' CATTGCCATGATTGGTCAAG 3': SEQ ID NO: 31); and
Primer wSS3pd (5' ACCACCTGTCCGTTCCGTTGC 3': SEQ ID NO: 32).

20 The amplified clones wSSIIIp1 and wSSIIIp2 were used as probes to screen the third cDNA library and *T. tauschii* genomic DNA library as described in Example 4.

A further probe designated wSSIIIp3 was used for screening the third cDNA library, as described in Example 4. Probe wSSIIIp3 was amplified by PCR from a cDNA clone
25 produced from the first screening using the following amplification primers:
Primer wSS3pe (5' GCACGGTCTATGAGAACAATGGC 3': SEQ ID NO: 33); and
Primer wSS3pf (5' TCTGCATACCACCAATCGCCG 3': SEQ ID NO: 34).

The amplification reactions were performed using a FTS-1 or FTS4000 thermal
30 sequencer (Corbett, Australia) for 1 cycle of 95°C for 2 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 1 minutes, 72°C for 2 minutes and 1 cycle of 25°C for 1 minute.

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Amplified sequences of the expected length were obtained, cloned and sequenced, and shown to contain DNA sequences highly homologous to the maize and potato SSIII genes. PCR fragments were subsequently used to probe a wheat cDNA library
5 by DNA hybridisation and 8 positive clones were obtained, including one 3 kb cDNA. A region from the 5' end of this cDNA was amplified by PCR and used a probe for a second round of screening the cDNA library, obtaining 8 cDNA clones. Of these, one cDNA was demonstrated to be full length (wSSIII.B3, 5.36 kb insert). The sequence of the 5,346 bp wSSIII.B3 cDNA clone is given in SEQ ID NO:7.

10

Sequencing of the 8 cDNA clones obtained from the second round screening of the wheat cDNA library revealed that there were at least 2 classes of cDNA encoding SSIII present, possibly being encoded by homeologous genes on different wheat genomes. The sequence of a representative of this second class of cDNA clones, wSSIII.B1, is
15 shown in SEQ ID NO:9. The 3261 bp clone wSSIII.B1 is not full length, however it is similar to nucleotides 1739 to 5346 of the homeologous clone wSSIII.B3 (SEQ ID NO: 7). Clone wSSIII.B1 has an open reading frame between nucleotide positions 1 and 3177.

20 An open reading frame is found in the cDNA clone wSSIII.B3 (SEQ ID NO:7), in the region between position 29, commencing the ATG start codon, and nucleotide position 4912. The amino acid sequence deduced from this open reading frame is shown in SEQ ID NO:8.

25 An alignment of the deduced amino acid sequences of SSIII from maize, potato and wheat is shown in Figure 7. There is about 56.6% identity between the maize SSIII and wheat wSSIII.B3 sequence at the amino acid level.

The C-terminal domain of starch synthases comprise the catalytic domain, and a
30 characteristic amino acid sequence motif KVGGLGDVVTSLSRVQDLGHNVEV (SEQ ID NO: 35) in maize, or alternatively KVGGLGDVVTSLSRAIQDLGHTVEV (SEQ ID

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NO: 36) in wheat, marking the first conserved region in the C-terminal domain. This amino acid sequence is present at amino acid residues 1194 to 1218 of SEQ ID NO: 8.

5 The amino acid identity between maize dull1 and wSSIII.B3 in the N-terminal region (i.e. amino acids 1 to 600 in Figure 7) is only 32.2%; whilst the amino acid identity in the central region (i.e. amino acids 601 to 1248 in Figure 7) is 68.4%; and in the C-terminal region (i.e. amino acids 1249 to 1631 in Figure 7) is 84.6%. Accordingly, the SSIII starch synthases are much more highly conserved between maize and wheat in
10 the region comprising the catalytic domain of the proteins.

EXAMPLE 14

Analysis of wheat starch synthase III mRNA expression

Figure 8 shows the expression of wSSIII mRNA during endosperm development in two
15 wheat varieties grown under defined environmental conditions. The expression of the gene is seen very early in endosperm development in both cultivars, 4 days after anthesis (Figure 8, panels a and b). Expression in the leaf of the variety Gabo is very weak (Figure 8, panel c, Lane L) whereas strong expression is seen in pre-anthesis florets (Figure 8, panel c, Lane P).

20

EXAMPLE 15

Amino acid sequence comparisons between wheat SSII and SSIII polypeptides

Amino acid sequence comparisons between wheat BSSS, SSI, SSII and SSIII
25 polypeptides reveals eight highly-conserved domains (Figure 9). The amino acid sequences of these domains are represented in the wheat SSIII amino acid sequence by the following sequence motifs:

- (a) Region 1: KVGGLGDVVT;
- (b) Region 2: GHTVEVILPKY;
- 30 (c) Region 3: HDWSSAPVAWLYKEHY;
- (d) Region 4: GILNGIDPDIWDPYTD;

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- (e) Region 5: DVPIVGIITRLTAQKG;
- (f) Region 5a: NGQVVLLGSA;
- (g) Region 6: AGSDFIIVPSIFEPGLTQLVAMRYGS; and
- (h) Region 7: TGGLVDTV.

5

These conserved amino acid sequences are summarised in Table 4. As shown in Table 4 below, there is at least about 25% amino acid sequence identity, preferably at least about 30% amino acid sequence identity, more preferably at least about 35% amino acid sequence identity, more preferably at least about 40% amino acid sequence identity, more preferably at least about 45% amino acid sequence identity, more preferably at least about 50% amino acid sequence identity, more preferably at least about 55% amino acid sequence identity, more preferably at least about 60% amino acid sequence identity, more preferably at least about 65% amino acid sequence identity, more preferably at least about 70% amino acid sequence identity, more preferably at least about 75% amino acid sequence identity, more preferably at least about 80% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity and even more preferably at least about 95% amino acid sequence identity between the amino acid sequences of plant starch synthase enzymes, in particular wheat starch synthases.

From the data presented in Table 4, the most conserved regions of the wheat SSII and SSIII polypeptides are a region of 6 or 7 identical amino acids in Region 1 and a region of 8 or 9 identical amino acids in Region 6. The lowest regions of identity are found in regions 3 and 5a.

For each of the amino acid sequences presented in the first column of Table 4, which are specific for wSSIII polypeptides, corresponding signature motifs which are specific for wSSII-A, wSSII-B, and wSSII-D polypeptides can be derived from the alignment, as follows:

Region 1: KTGGLGDVAGA;

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- Region 2: GHRVMVVVPRY;
- Region 3: NDWHTALLPVYLKAYY;
- Region 4: GIVNGIDNMEWNPEVD;
- Region 5: DVPLLGFIGRLDGQKG;
- 5 Region 5a: DVQLVMLGTG;
- Region 6: AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- Region 7: VGG(V/L)RDTV.

Comparison of the amino acid sequences of all available starch synthases with the
 10 deduced amino acid sequences of the three wSSII cDNA clones of the present
 invention (i.e. wSSIIB, wSSIIA and wSSIID) was conducted using PILEUP analysis
 (Devereaux *et al.*, 1984) and data are presented herein as a dendrogram (Figure 10).
 The sequence of the glycogen synthase of *E. coli* was also included. Based upon their
 amino acid similarities, four classes of plant starch synthases can be defined: GBSS,
 15 SSI, SSII and SSIII.

Table 5 shows that levels of identity at the amino acid level between the wSSII
 sequences, as determined using the BESTFIT programme in GCG (Devereaux *et al.*,
 1984), and other class II starch synthases range from 70% identity with potato SSII to
 20 85% identity with maize SSIIa. Both wSSIIB and wSSIID showed significantly higher
 homology to maize SSIIa than wSSIIA. Based upon sequence identities and the
 function of the Sgp-1 proteins in wheat, the wSSIIB, wSSIIA and wSSIID cDNA clones
 are members of the starch synthase II (SSII) group and are more similar in sequence
 to maize SSIIa than maize SSIIb.

25

TABLE 4**Identities between conserved motifs of plant starch synthases**

	Sequence in wSSIII polypeptide	Number of conserved residues between wheat starch synthases	Number of conserved residues between wheat SSII and SSIII polypeptides
5	Region 1: KVGGLGDVVT	6/11 residues	6/11 residues
	Region 2: GHTVEVILPKY	6/11 residues	6/11 residues
10	Region 3: HDWSSAPVAWLYKEHY	4/16 residues	5/16 residues
	Region 4: GILNGIDPDIWDPYTD	7/16 residues	8/16 residues
	Region 5: DVPIVGIIITRLTAQKG	8/16 residues	10/16 residues
15	Region 5a: NGQVVLLGSA	4/10 residues	4/10 residues
	Region 6: AGSDFIIVPSIFPCGLT QLVAMRYGS	15/27 residues	17/27 residues
20	Region 7: TGGLVDTV	5/9 residues	5/9 residues

TABLE 5

	wSSII-A	wSSII-B	wSSII-D
wSSI-A	100%		
wSSII-B	95.9%	100%	
5 wSSII-D	96.3%	96.7%	100%
maize SSIIa	76.1%	85.2%	84.7%
maize SSIIb	76.3%	76.7%	75.9%
pea SSII	72.0%	72.2%	71.8%
10 potato SSII	70.9%	71.1%	70.3%

Figure 11 shows a schematic representation of an alignment of plant starch synthase sequences, including wheat GBSS, wheat SSI, wheat SSII-A1, maize SSIIa, and maize dull-1 polypeptides, in which the position of the first homologous region, comprising the consensus motif KXGG, is used as the basis of the alignment. The major differences in structure between the classes of genes are found in the length of the N-terminal region between the transit peptide and the first conserved region. At one extreme, the GBSS genes have a very short N-terminal arm, whereas the *du1* starch synthase contains a very long N-terminal extension containing several distinct regions. The wSSII genes contain an N-terminal extension which is longer than either GBSS, SSI, or SSIIb, and slightly longer than the maize SSIIa gene.

EXAMPLE 16

Isolation of genomic clones for SSIII

Screening of a genomic library from the D-genome donor of wheat, *T. tauschii*, identified a number of clones which hybridised to the wSSIII PCR fragment. Positive plaques in the genomic library were selected as those hybridising with a probe that had been generated by PCR (amplifying between nucleotide positions 3620 to 3966) from the SSIII cDNA as template. The primer sequences used were as follows:

wSS3pa (5' GGAGGTCTTGGTGATGTTGT 3': SEQ ID NO: 29); and
 30 wSS3pb (5' CTTGACCAATCATGGCAATG 3' : SEQ ID NO: 30).

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Hybridisation was carried out in 25% formamide, 6 x SSC, 0.1% SDS at 42 °C for 16 hour, then washed three times with 2 x SSC containing 0.1% SDS at 65 °C, for 1 hour per wash. shows an example of a plaque lift showing positive and negative hybridisations for plaques containing the *T. tauschii* homologue of the wSSIII.B3 gene.

5

DNA was isolated from positive-hybridising λ clones using methods described by Maniatis *et al.* Briefly, DNA was digested using *Bam*HI or *Bgl*II and sub-cloned in to the vector pJKKmfm. DNA sequencing was performed using the automated ABI system with dye terminators as described by the manufacturers. DNA sequences were
10 analysed using the GCG suite of programs (Devereaux *et al.*, 1984).

Nucleotide sequences of the genomic SSIII clone from *T. tauschii* are provided herein as 6 contiguous sequences designated fragments 1 to 6 (SEQ ID NOs: 11 to 16, respectively). Table 6 defines the relative positions of these fragments with respect to
15 the SSIII cDNA and describes the positions of exons. Figure 11 shows this information schematically.

The complete nucleotide sequence of a wheat SSIII genomic gene is presented herein as SEQ ID NO: 38. The structural features of this gene are presented in Table 7. A
20 schematic representation of the intron/exon organisation of this gene is also presented in Figure 12.

EXAMPLE 17

Discussion

25 Early work on the Sgp-1 starch synthase proteins (Denyer *et al.*, 1995; Rahman *et al.*, 1995) was based on the localisation of these proteins in the wheat starch granule, and no definitive conclusion concerning their presence or absence in soluble extracts of the wheat endosperm was presented.

30 We have now demonstrated that a monoclonal antibody against the Sgp-1 proteins cross reacts strongly with those starch synthase proteins having apparent molecular

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weights of 100-105 kDa in soluble extracts, however, the appearance of these proteins in soluble extracts is dependant on the developmental stage of the endosperm material. Whilst the proteins can be detected in the soluble phase in early to mid endosperm development, little or no soluble protein remains in late endosperm development (Figure 1). This observation accounts for the failure of Rahman *et al.* (1995) to detect the protein in soluble extracts in a previous report.

Based upon the localisation of the Sgp-1 starch synthase proteins in the wheat endosperm, the following nomenclature is suggested for wheat starch synthase enzymes: wGBSS for the 60 kDa granule bound starch synthase (Wx); wSSI for the 75 kDa starch synthase I (Sgp-3); wSSII for the 100 - 105 kDa proteins (Sgp-1); and wSSIII for a soluble high molecular starch synthase.

The present invention provides cDNA and genomic clones encoding the wSSII and wSSIII polypeptides and the corresponding genomic clones. Whilst the evidence is compelling that the wSSIIA, wSSIIB and wSSIID cDNAs encode the Sgp-A1, Sgp-B1 and Sgp-D1 proteins of the wheat starch granule, molecular weights calculated from the deduced amino acid sequences of the clones are considerably lower than estimates obtained from SDS-PAGE. The molecular weight of the precursor wSSIIA protein is 87,229 Da, and the mature protein 81,164 Da, yet the estimated molecular weight in our experience is 105 kDa. The assignment of the wSSIIA cDNA to the A-genome of wheat is demonstrated in Figure 5, and the assignment of the 105 kDa protein to the A-genome in Denyer *et al.* (1995) and Yamamori and Endo (1996). Similarly, the molecular weight of the wSSIIB protein is 86,790 Da and the mature protein 80,759 Da, yet the molecular weight of the Sgp-B1 protein is estimated to be 100 kDa. No comparison can be made of the wSSIID sequences as a full length cDNA clone was not obtained. The wSSIIA and wSSIIB amino acid sequences differ by just a single amino acid residue, yet there is an apparent difference of 5 kDa in molecular weight when estimated by SDS-PAGE. Several possibilities can be advanced to account for this apparent discrepancy in molecular weights. Firstly, the wSSII proteins may not migrate in SDS-PAGE in accordance with their molecular weight because they

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retain some conformation under the denaturing conditions used. Secondly, the proteins may be glycosylated. It is also possible that the proteins may be non-covalently linked to starch through a high affinity starch binding site which survives denaturation and SDS-PAGE. Differences between the apparent molecular weights and those calculated
5 from the deduced amino acid sequences will have to be defined in establishing the relationship between the wSSII proteins and proteins encoded by the analogous SSII genes of other species.

The catalytic domain of the starch synthases is found at the C-terminal end of the
10 protein (Gao *et al.*, 1998; Harn *et al.*, 1998). Harn *et al.* (1998) identified 7 conserved regions among SSIIa, SSIIb, SSII and GBSS sequences. We have identified an additional conserved region (designated region 5a in Table 4 and Figure 10) comprising the amino acid sequence motif DVQLVMLGTG, by a comparison of the wSSII and wSSIII sequences of the present invention with differing isoforms of other
15 plant starch synthases (GBSS, SSII, SSII and SSIII). The conservation of eight peptide regions among the 4 classes of starch synthases is striking, in terms of their sequence homologies and their alignment.

Analysis of the wheat SSII genes shows that there is a motif, PVNGENK, which is
20 repeated. The area surrounding the repeated PVNGENK motif is not homologous to maize SSIIa and the insertion of this region is responsible for the difference in length between the wheat SSII and maize SSIIa genes. In pea and potato SSII polypeptides, a PPP motif (Figure 3; residues 251-253 and 287-289 respectively) has been suggested to mark the end of the N-terminal region and to facilitate the flexibility of an
25 "N-terminal arm". This motif is not found in either the maize or wheat SSII sequences.

The generation of a wheat line combining null alleles at each of the three wSSII loci, wSSIIA, wSSIIB and wSSIID, has been reported recently by Yamamori (1998). In this triple null line, the large starch granules were reported to be mostly deformed and a
30 novel starch with high blue value was observed when stained with iodine, indicating that wSSII is a key enzyme for the synthesis of starch in wheat. Further analysis of the

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starch derived from this triple null mutant is in progress.

Mutations in starch synthases are known in three other species. In pea, mutation in SSII gives rise to starch with altered granule morphology and an amylopectin which yields an oligosaccharide distribution with reduced chain length on debranching, compared to the wild type (Craig *et al.*, 1998). A similar mutation in a gene designated SSII is known in *Chlamydomonas* (the *sta-3* mutation) and similar effects on granule morphology and amylopectin structure are observed (Fontaine *et al.*, 1993). In maize, two mutations affecting starch synthases are known. First, the *dull1* mutation has been shown to be caused by a lesion within the *du1* SSIII-type starch synthase gene (Gao *et al.*, 1998). A second mutation, the *sugary-2* mutation yields a starch with reduced amylopectin chain lengths on debranching (this mutation co-segregates with the SSIIa locus (Harn *et al.*, 1998) although direct evidence that the *sugary-2* mutation is caused by a lesion in the SSIIa gene is lacking). In the SSII mutants of each of these species, amylose biosynthesis capacity is retained, suggesting different roles in amylose and amylopectin synthesis for the GBSS and SSII genes. Given the conservation in overall organisation of the GBSS and SSII genes (see Figures 12 and 13), when an alignment is made based on the KTGGL motif of the first conserved region, this focuses attention on the role(s) of the N-terminal region in defining substrate specificity and the localisation of the proteins as the N-terminal region is the major area of divergence between the 4 classes of starch synthases. However, it is premature to exclude the influence of more subtle mutations in central and C-terminal regions of the gene.

The cloning of the wSSII and wSSIII cDNAs and genomic clones described herein provides useful tools for the further study of the roles of the starch synthases in wheat. Firstly, they provide a source of markers which can be used to recover and combine null or divergent alleles. Secondly, genetic manipulation of wheat by gene suppression or over-expression can be carried out, and the genes may be used for over expression in other species. The promoter regions of these genes are also useful in regulating the expression of starch synthase genes and other heterologous genes in the developing wheat endosperm and in pre-anthesis florets of wheat.

TABLE 6
Summary of the Wheat Starch Synthase III Genomic Sequence

Fragment in genomic DNA clone	Length (bp)	Features in SEQ ID NOS:11 to 16	Corresponding region in cDNA sequence
Fragment 1 (SEQ ID NO: 11)	728	Translation start codon (nucleotides 287 to 289); Exon 1.1 (nucleotides 260 to 385).	Exon 1.1: nucleotides 1 to 126
Fragment 2 (SEQ ID NO: 12)	2446	Exon 2.1 (nucleotides 1 to 1938); Exon 2.2 (nucleotides 2197 to 2418).	Exon 2.1: nucleotides 1008 to 2948; Exon 2.2: nucleotides 2949 to 3171
Fragment 3 (SEQ ID NO: 13)	1032	Exon 3.1 (nucleotides 310 to 580)	Exon 3.1: nucleotides 3172 to 3440
Fragment 4 (SEQ ID NO: 14)	892	Exon 4.1 (nucleotides 678 to 853)	Exon 4.1: nucleotides 3441 to 3616
Fragment 5 (SEQ ID NO: 15)	871	Partial Exon 5.1 (nucleotides 1 to 29) Exon 5.2 (nucleotides 293 to 463) Exon 5.3 (nucleotides 589 to 695)	Exon 5.1: nucleotides 3908 to 3937 (partial) Exon 5.2: nucleotides 3938 to 4108 Exon 5.3: nucleotides 4109 to 4215
Fragment 6 (SEQ ID NO: 16)	1583	Exon 6.1 (nucleotides 471 to 653); Exon 6.2 (nucleotides 770 to 902); Exon 6.3 (nucleotides 999 to 1110); Exon 6.4 (nucleotides 1201 to 1328); Partial Exon 6.5 (nucleotides 1408 to 1583); Translation stop codon (nucleotides 1536 to 1538)	Exon 6.1: nucleotides 4238 to 4420 Exon 6.2: nucleotides 4421 to 4552 Exon 6.3: nucleotides 4553 to 4664 Exon 6.4: nucleotides 4665 to 4793 Exon 6.5: nucleotides 4794 to 4966 (partial)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

TABLE 7
Structural features of the wheat starch synthase III genomic gene

	Nucleotide Position in SEQ ID NO: 38	Feature	Length (bases)
5	1- 973	5'-untranscribed region and promoter sequence	973
	974 - 1099	exon 1	126
	1001-1003	translation start codon (ATG)	3
	1100 - 2056	intron 1	957
	2057 - 2120	exon 2	64
10	2121 - 2588	intron 2	468
	2589 - 5291	exon 3	2703
	5292 - 5549	intron 3	258
	5550 - 5767	exon 4	218
	5768 - 6103	intron 4	336
15	6104 - 6374	exon 5	271
	6375 - 7148	intron 5	774
	7149 - 7324	exon 6	176
	7325 - 7438	intron 6	114
	7439 - 7546	exon 7	108
20	7547 - 7792	intron 7	246
	7793 - 7902	exon 8	110
	7903 - 8797	intron 8	895
	8798 - 8900	exon 9	103
	8901 - 9164	intron 9	264
25	9165 - 9335	exon 10	171
	9336 - 9460	intron 10	125
	9461 - 9589	exon 11	129
	9590 - 9677	intron 11	88

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	9678 - 9860	exon 12	183
	9861 - 9977	intron 12	117
	9978 - 10109	exon 13	132
	10110 - 10205	intron 13	96
5	10206 - 10317	exon 14	112
	10318 - 10407	intron 14	90
	10408 - 10536	exon 15	129
	10537 - 10618	intron 15	82
	10619 - 11146	exon 16	128
10	10744 - 10746	translation stop codon (TGA)	3
	11147 - 11611	3'-untranscribed region	465

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CLAIMS:

1. An isolated nucleic acid molecule which comprises a sequence of nucleotides selected from the group consisting of:
 - (i) the nucleotide sequence set forth in SEQ ID NO: 1 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (ii) the nucleotide sequence set forth in SEQ ID NO: 3 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (iii) the nucleotide sequence set forth in SEQ ID NO: 5 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (iv) the nucleotide sequence set forth in SEQ ID NO: 7 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (v) the nucleotide sequence set forth in SEQ ID NO: 9 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (vi) the nucleotide sequence set forth in SEQ ID NO: 11 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (vii) the nucleotide sequence set forth in SEQ ID NO: 12 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (viii) the nucleotide sequence set forth in SEQ ID NO: 13 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (ix) the nucleotide sequence set forth in SEQ ID NO: 14 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (x) the nucleotide sequence set forth in SEQ ID NO: 15 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (xi) the nucleotide sequence set forth in SEQ ID NO: 16 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (xii) the nucleotide sequence set forth in SEQ ID NO: 37 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
 - (xiii) the nucleotide sequence set forth in SEQ ID NO: 38 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

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region thereof or a degenerate nucleotide sequence thereto;

(xiv) the nucleotide sequence set forth in SEQ ID NO: 11 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(xv) a nucleotide sequence which encodes a wheat starch synthase polypeptide as hereinbefore defined wherein said nucleotide sequence has at least about 85% identity overall to any one of (i) to (xiv); and

(xvi) a nucleotide sequence which is complementary to any one of (i) to (xv).

2. The isolated nucleic acid molecule according to claim 1 wherein the wheat starch synthase polypeptide further comprises one or more amino acid sequences selected from the group consisting of:

- (a) KVGGLGDVVT;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIIITRLTAQKG;
- (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFPCGLTQLVAMRYGS;
- (h) TGGLVDTV;
- (i) KTGGLGLVAGA;
- (j) GHRVMVVVPRY;
- (k) NDWHTALLPVYLKAYY;
- (l) GIVNGIDNMEWNPEVD;
- (m) DVPLLGFGRDLDGQKG;
- (n) DVQLVMLGTG;

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(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(p)VGG(V/L)RDTV.

3. The isolated nucleic acid molecule according to claim 2 wherein the wheat starch synthase polypeptide comprises at least three of said amino acid sequences selected from the group consisting of (a) to (h).
4. The isolated nucleic acid molecule according to claim 2 wherein the wheat starch synthase polypeptide comprises at least six of said amino acid sequences selected from the group consisting of (i) to (p).
5. The isolated nucleic acid molecule according to claim 1 encoding a wheat starch synthase II polypeptide.
6. The isolated nucleic acid molecule according to claim 1 encoding a wheat starch synthase III polypeptide.
7. An isolated nucleic acid molecule encoding a starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:
 - (a) GHTVEVILPKY;
 - (b) HDWSSAPVAWLYKEHY;
 - (c) DVPIVGIITRLTAQKG;
 - (d) NGQVVLLGSA;
 - (e) AGSDFIIVPSIFEPGLTQLVAMRYGS;
 - (f) TGGLVDTV;
 - (g) GIVNGIDNMEWNPEVD; and

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(h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.

8. The isolated nucleic acid molecule of claim 5 encoding a wheat starch synthase II polypeptide which comprises an amino acid sequence selected from the group consisting of:
 - (i) SEQ ID NO: 2;
 - (ii) SEQ ID NO: 4;
 - (iii) SEQ ID NO: 6; and
 - (iv) a homologue of any one of (i) to (iii) having at least about 85% identity thereto.
9. The isolated nucleic acid molecule of claim 6 encoding a wheat starch synthase III polypeptide which comprises an amino acid sequence selected from the group consisting of:
 - (i) SEQ ID NO: 8;
 - (ii) SEQ ID NO: 10; and
 - (iii) a homologue of (i) or (ii) having at least about 85% identity thereto.
10. A probe or primer comprising at least about 15 contiguous nucleotides in length derived from the nucleotide sequence according to claim 1.
11. The probe or primer according to claim 10 comprising a nucleotide sequence selected from the group consisting of:
 - (i) the nucleotide sequence set forth in SEQ ID NO: 25;
 - (ii) the nucleotide sequence set forth in SEQ ID NO: 26;
 - (iii) the nucleotide sequence set forth in SEQ ID NO: 27;

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- (iv) the nucleotide sequence set forth in SEQ ID NO: 28;
- (v) the nucleotide sequence set forth in SEQ ID NO: 29;
- (vi) the nucleotide sequence set forth in SEQ ID NO: 30;
- (vii) the nucleotide sequence set forth in SEQ ID NO: 31;
- (viii) the nucleotide sequence set forth in SEQ ID NO: 32;
- (ix) the nucleotide sequence set forth in SEQ ID NO: 33;
- (x) the nucleotide sequence set forth in SEQ ID NO: 34;
- (xi) a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of:

- (a) KVGGLGDVVTs;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIITRLTAQKG;
- (f) NGQVLLGSA;
- (g) AGSDFIIVPSIFPCGLTQLVAMRYGS;
- (h) TGGLVDTV;
- (i) KTGGLGDVAGA;
- (j) GHRVMVVVPRY;
- (k) NDWHTALLPVYLKAYY;
- (l) GIVNGIDNMEWNPEVD;
- (m) DVPLLGFGRLDGQKG;
- (n) DVQLVMLGTG;
- (o) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

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(p)VGG(V/L)RDTV;

- (xii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 37;
- (xiii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 38; and
- (xiv) a nucleotide sequence which is complementary to any one of (i) to (xiii).

12. An isolated or recombinant polypeptide, protein or enzyme comprising an amino acid sequence selected from the following:

- (i) the amino acid sequence set forth in SEQ ID NO: 2 or the mature protein region thereof;
- (ii) the amino acid sequence set forth in SEQ ID NO: 4 or the mature protein region thereof;
- (iii) the amino acid sequence set forth in SEQ ID NO: 6 or the mature protein region thereof;
- (iv) the amino acid sequence set forth in SEQ ID NO: 8 or the mature protein region thereof;
- (v) the amino acid sequence set forth in SEQ ID NO: 10 or the mature protein region thereof;
- (vi) a wheat starch synthase polypeptide having at least about 85% identity overall to any one of (i) to (v).

13. The isolated or recombinant polypeptide according to claim 12 further comprising one or more amino acid sequences selected from the group consisting of:

- (a) KVGGLGDVVT;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;

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(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVVLLGSA;

(g) AGSDFIIVPSIFPCGLTQLVAMRYGS;

(h) TGGLVDTV;

(i) KTGGLGDVAGA;

(j) GHRVMVVVPRY;

(k) NDWHTALLPVYLKAYY;

(l) GIVNGIDNMEWNPEVD;

(m) DVPLLGFIRLDGQKG;

(n) DVQLVMLGTG;

(o) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(p) VGG(V/L)RDTV.

14. The isolated or recombinant polypeptide according to claim 13 wherein the wheat starch synthase polypeptide comprises at least three of said amino acid sequences selected from the group consisting of (a) to (h).
15. The isolated or recombinant polypeptide according to claim 13 wherein the wheat starch synthase polypeptide comprises at least six of said amino acid sequences selected from the group consisting of (i) to (p).
16. The isolated or recombinant polypeptide according to claim 12 encoding a wheat starch synthase II polypeptide.

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17. The isolated or recombinant polypeptide according to claim 12 encoding a wheat starch synthase III polypeptide.
18. An isolated or recombinant starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:
 - (a) GHTVEVILPKY;
 - (b) HDWSSAPVAWLYKEHY;
 - (c) DVPIVGIIITRLTAQKG;
 - (d) NGQVVLLGSA;
 - (e) AGSDFIIVPSIFPCGLTQLVAMRYGS;
 - (f) TGGLVDTV;
 - (g) GIVNGIDNMEWNPEVD; and
 - (h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.
19. The isolated or recombinant polypeptide according to claim 16 consisting of a wheat starch synthase II polypeptide which comprises an amino acid sequence selected from the group consisting of:
 - (i) SEQ ID NO: 2;
 - (ii) SEQ ID NO: 4;
 - (iii) SEQ ID NO: 6; and
 - (iv) a homologue of any one of (i) to (iii) having at least about 85% identity thereto.
20. The isolated or recombinant polypeptide according to claim 17 consisting of a wheat starch synthase III polypeptide which comprises an amino acid sequence selected from the group consisting of:

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- (i) SEQ ID NO: 8;
 - (ii) SEQ ID NO: 10; and
 - (iii) a homologue of (i) or (ii) having at least about 85% identity thereto.
- 21. The isolated or recombinant polypeptide according to claim 12 substantially free of conspecific or non-specific proteins.
- 22. A method comprising:
 - (i) hybridising single-stranded or double-stranded mRNA, cDNA or genomic DNA with a nucleotide sequence selected from the group consisting of:
 - (a) the nucleotide sequence according to any one of claims 1 to 9;
 - (b) a probe or primer derived from a nucleotide sequence according to subparagraph (a) and comprising at least about 15 contiguous nucleotides of said nucleotide sequence in length; and
 - (ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.
- 23. The method according to claim 22 wherein the detecting means consists of a reporter molecule covalently attached to the probe or primer molecule.
- 24. The method according to claim 22 wherein the detecting means consists of a polymerase chain reaction.
- 25. The method according to claim 22 wherein the probe or primer comprises a nucleotide sequence selected from the group consisting of:
 - (i) the nucleotide sequence set forth in SEQ ID NO: 25;

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- (ii) the nucleotide sequence set forth in SEQ ID NO: 26;
- (iii) the nucleotide sequence set forth in SEQ ID NO: 27;
- (iv) the nucleotide sequence set forth in SEQ ID NO: 28;
- (v) the nucleotide sequence set forth in SEQ ID NO: 29;
- (vi) the nucleotide sequence set forth in SEQ ID NO: 30;
- (vii) the nucleotide sequence set forth in SEQ ID NO: 31;
- (viii) the nucleotide sequence set forth in SEQ ID NO: 32;
- (ix) the nucleotide sequence set forth in SEQ ID NO: 33;
- (x) the nucleotide sequence set forth in SEQ ID NO: 34;
- (xi) a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of:

- (a) KVGGLGDVVT;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIITRLTAQKG;
- (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS;
- (h) TGGLVDTV;
- (i) KTGGLGDVAGA;
- (j) GHRVMVVVPRY;
- (k) NDWHTALLPVYLKAYY;
- (l) GIVNGIDNMEWNPEVD;
- (m) DVPLLGFGRLDGQKG;

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(n) DVQLVMLGTG;

(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(p)VGG(V/L)RDTV;

- (xii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 37;
- (xiii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 38; and
- (xiv) a nucleotide sequence which is complementary to any one of (i) to (xiii).

- 26. A method of assaying for the presence or absence of a wheat starch synthase polypeptide in a plant or a plant extract or isolated nucleic acid sample, said method at least comprising performing the method according to any one of claims 22 to 25.
- 27. The method according to claim 26 further comprising preparing the plant extract or nucleic acid sample.
- 28. A method of marker-assisted breeding and/or selection of a plant at least comprising performing the method according to any one of claims 22 to 25.
- 29. The method according to claim 28 further comprising selecting a plant which expresses a desirable wheat starch synthase characteristic.
- 30. The method according to claim 28 further comprising crossing a plant which expresses a desirable wheat starch synthase characteristic to another plant.
- 31. The method according to claim 30 further comprising selecting progeny of the cross

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which expresses a desirable wheat starch synthase characteristic.

32. A plant produced by the method according to any one of claims 28 to 31 wherein said plant expresses a wheat starch synthase polypeptide at a desired level detectable using said method.
33. A method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing in said plant a nucleic acid molecule for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified, wherein said nucleic acid molecule is selected from the group consisting of:
 - (i) the isolated nucleic acid molecule according to any one of claims 1 to 9;
 - (ii) a fragment of (i) which comprises a nucleotide sequence capable of being expressed to down-regulate the expression of an endogenous wheat starch synthase isoenzyme of said plant; and
 - (iii) a fragment of (i) which encodes a functional wheat starch synthase isoenzyme of said plant.
34. The method according to claim 33 wherein the fragment at sub-paragraph (ii) is an antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule.
35. The method according to claim 33 further comprising introducing the nucleic acid molecule to an isolated plant cell, tissue, organ, or organelle.
36. The method according to claim 35 further comprising regenerating an intact plant from the isolated plant cell, tissue, organ, or organelle carrying the introduced nucleic acid molecule.

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37. The method according to claim 35 wherein the nucleic acid molecule is introduced to the plant cell, tissue, organ, or organelle by introgression.
38. The method according to claim 35 wherein the nucleic acid molecule is introduced to the plant cell, tissue, organ, or organelle by transformation means.
39. An isolated promoter sequence comprising a nucleotide sequence selected from the group consisting of:
 - (i) nucleotides 1 to about 287 of SEQ ID NO: 11;
 - (ii) nucleotides 1 to about 1416 of SEQ ID NO: 37;
 - (iii) nucleotides 1 to about 973 of SEQ ID NO: 38;
 - (iv) a fragment of any one of (i) to (iii) capable of conferring expression on a heterologous gene in a monocotyledonous plant cell, tissue or organ; and
 - (v) a complementary nucleotide sequence to any one of (i) to (iv).
40. The isolated promoter sequence according to claim 39 that is operable in the endosperm.
41. A plant carrying the isolated nucleic acid molecule according to any one of claims 1 to 9 as an exogenous complement to its genome.
42. A progeny of the plant according to claim 41 wherein said progeny carries the introduced nucleic acid molecule.
43. A propagule of the plant according to claim 41 or 42 wherein said propagule carries the

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introduced nucleic acid molecule present in said plant.

44. A gene construct or vector which comprises the isolated nucleic acid molecule according to any one of claims 1 to 9 and one or more origins of replication.
45. The gene construct according to claim 44 further comprising a promoter sequence in operable connection with said isolated nucleic acid molecule.
46. A gene construct or vector which comprises the probe or primer according to claim 10 or 11 and one or more origins of replication.
47. A modified starch derived from the plant according to claim 32 or 41 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.
48. A modified starch derived from the progeny according to claim 42 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said progeny.
49. A modified starch derived from the propagule according to claim 43 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said propagule.
50. A food product comprising the modified starch according to any one of claims 47 to 49.
51. The food product according to claim 50 consisting of flour or a flour-based food product.

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52. The food product according to claim 50 or 51 selected from the group consisting of: flour-based sauce; leavened bread; unleavened bread; pasta, noodle; cereal; snack food; cake; and pastry.
53. Use of the modified starch according to any one of claims 47 to 49 in the preparation of a food product for consumption by an animal or human.
54. A modified protein derived from the plant according to claim 32 or 41 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.
55. A modified protein derived from the progeny according to claim 42 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said progeny.
56. A modified protein derived from the propagule according to claim 43 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said propagule.
57. A non-food product comprising the modified protein according to any one of claims 54 to 56.
58. The non-food product according to claim 57 selected from the group consisting of: films; coatings; adhesives; building materials; and packaging materials.
59. Use of the modified protein according to any one of claims 54 to 56 in the preparation of a non-food product.

PCT

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(21) International Application Number: PCT/AU00/00385 (22) International Filing Date: 28 April 2000 (28.04.00) (30) Priority Data: PQ0052/99 29 April 1999 (29.04.99) AU (71) Applicants (for all designated States except US): COMMON-WEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; Limestone Avenue, Campbell, Australian Capital Territory 2601 (AU). GOODMAN FIELDER LIMITED [AU/AU]; Level 42 Grosvenor Place, Sydney, New South Wales 2000 (AU). GROUPE LIMAGRAIN PACIFIC PTY LTD [AU/AU]; Level 31, 1 O'Connell Street, Sydney, New South Wales 2000 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): MORELL, Matthew [AU/AU]; 33 Wangara Street, Aranda, Australian Capital Territory 2614 (AU). LI, Zhongyi [AU/AU]; 63 Campaspe Circuit, Kaleen, Australian Capital Territory 2617 (AU). RAHMAN, Sadequr [AU/AU]; 46 Scarlett Street, Melba, Australian Capital Territory 2615 (AU). APPELS, Rudolph [AU/AU]; 40 Gingara Street, Aranda, Australian Capital Territory 2614 (AU).		(74) Agents: OLIVE, Mark, R. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, Victoria 3000 (AU). (81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>	
(54) Title: NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES THEREFOR			
(57) Abstract <p>The present invention provides isolated nucleic acid molecules encoding wheat starch synthases, and probes and primers derived therefrom, which are useful in the modification of plant starch content and/or composition, and for screening plant lines to determine the presence of natural and/or induced mutations in starch synthase genes which affect starch content and/or composition. More particularly, the isolated nucleic acid molecules of the present invention further provide for the screening-assisted breeding of plants having desirable starch content and/or composition, in addition to providing for the direct genetic manipulation of plant starch content and/or composition.</p>			

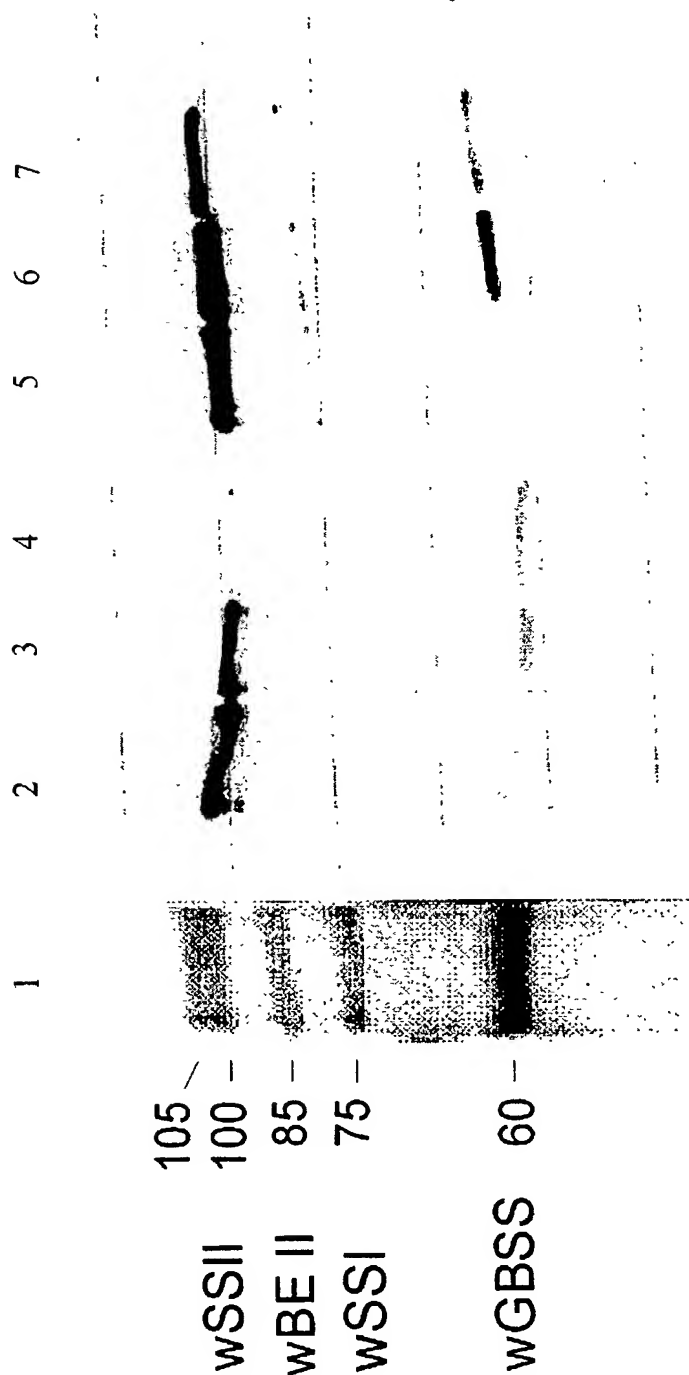


FIGURE 1

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FIGURE 2A
FIGURE 2B
FIGURE 2C
FIGURE 2D
FIGURE 2E
FIGURE 2F
FIGURE 2G
FIGURE 2H
FIGURE 2I
FIGURE 2J
FIGURE 2K
FIGURE 2L
FIGURE 2M
FIGURE 2N
FIGURE 2O

FIGURE 2

1 50
wSSIIB ATTCCTCGG CCTGACCCCG TCGGTTTACC CCACACAGAG CACACTCCAG
wSSIID ~~~~~~
wSSIIA ~~~~~~
51 100
wSSIIB TCCAGTCCAG CCCACTGCCG CGCTACTCCC CACTCCCACT GCCACCACCT
wSSIID ~~~~~~
wSSIIA ~~~~~~GCT GCCACCACCT
101 150
wSSIIB CCGCCTGCGC CGCGCTCTGG GCGGACCAAC CCGGCGATCG TATCAGCATC
wSSIID ~~~~~~
wSSIIA CCGCCTGCGC CGCGCTCTGG GCGGAGGACC AACCCGCGCA TCGTACCATC
151 200
wSSIIB ACCCACCCTCG ATCCCGGCCG CCGCCATGTC GTCGGCGGTC GCGTCCGCCG
wSSIID ~~~~~~
wSSIIA GCCCGCCCTCG ATCCCGGCCG CCGCCATGTC GTCGGCGGTC GCGTCCGCCG

FIGURE 2A


```
250
WSSIIB CGTCCTTCCT CGCGCTCGCG TCCGCCTCCC CCGGGAGATC ACGGAGGAGG
WSSIID ~~~~~~
WSSIIA CGTCCTTCCT CGCGCTCGCC TCCGCCTCCC CCGGGAGATC ACGCAGGCGG

300
WSSIIB ACGAGGGTGA GCGGCTCGCC ACCCCACACC GGGGCTGGCA GGTGCACTG
WSSIID ~~~~~~
WSSIIA GCGAGGGTGA GCGGCGCGCC ACCCCACGCC GGGGCCGGCA GGCTGCACTG

350
WSSIIB GCCGCCGTGG CCGCCGCAGC GCACGGCTCG CGACGGAGCG GTGGCCGCGC
WSSIID ~~~~~~
WSSIIA GCCGCCGTGG CCGCCGCAGC GCACGGCTCG CGACGGAGGT GTGGCCGCGC

400
WSSIIB GCGCCGCCGG GAAGAAGGAC GCGGGGAT.. CGACGACGC CGGCCCCGCG
WSSIID ~~~~~~
WSSIIA GCGCCGCCGG GAAGAAGGAC GCGAGGGTCG ACGACGACGC CGGTCCGCGG
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FIGURE 2B

5/50

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401      450
wSSIIB  AGGCAGCCCC GCGCACTCCG CCGTGGCGCC GCCACCAAGG TTGCGGAGCG
wSSIID  ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~
wSSIIA  AGGCAGCCCC GCGCACGCCG CCGTGGCGCC GcCACCAAGG TCGCGGAGCG

451      500
wSSIIB  GAGGGATCCC GTCAAGACGC TCGATCGCGA CGCCGCGGAA GGTGGCGCGC
wSSIID  ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~
wSSIIA  GAGGGATCCC GTCAAGACGC TCGATCGCGA CGCCGCGGAA GGTGGCGCGC

501      550
wSSIIB  CGTCCCCCGCC GGCACCGAGG CAGGAGGACG CCCGTCTGCC GAGCATGAAC
wSSIID  ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~
wSSIIA  CGGCACCGCC GGCACCGAGG CAGGACGCCG CCCGTCCaCC GAGTATGAAC

551      600
wSSIIB  GGCATGCCCG TGAACGGTGA AAACAAATCT ACCGGCGGCG GCGGCGCGAC
wSSIID  ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~
wSSIIA  GGCACGCCCG TGAACGGTGA GAACAAATCT ACCGGCGGCG GCGGCGCGAC
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FIGURE 2C

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601      650
wSSIIB  TAAAGACAGC GGGCTGCCCG CACCCGCACG CGCGCCCCAG CCGTCGAGCC
wSSIID  ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~
wSSIIA  CAAAGACAGC GGGCTgcCCG CACCCGcACG CGCGCCCCAT CCGTCGACCC

651      700
wSSIIB  AGAACAGAGT ACCGGTGAAT GGTGAAAACA AAGCTAACGT CGCCTCGCCG
wSSIID  ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~
wSSIIA  AgAACAgAGT ACCAGTGAAC GGTGAAAACA AAGCTAACGT CGCCTCGCCG

701      750
wSSIIB  CCGACGAGCA TAGCCGAGGT CGCGGGCTCCG GATCCCGCAG CTACCATTTT
wSSIID  ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~
wSSIIA  CCGACGAGCA TAGCCGAGGT CGTGGCTCCG GATTCCGCAG CTACCATTTT

751      800
wSSIIB  CATCAGTGAC AAGGCGCCAG AGTCCGTTGT CCCAGCCGAG AAGGgcgcg
wSSIID  ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~ ~~~~~~
wSSIIA  CATCAGTGAC AAGGCGCCGG AGTCCGTTGT CCCAGCCGAG AAGCCGCCG
```

FIGURE 2D

801
wSSIIB CGtCgtcCgg CtcAAATtTc gtgCcCtCgg cttctGctCc cggGtctGAC 850
wSSIID CGTCGTCCGG CTCAAATtTc GAGTCCTCGG CCTCTGCTCC CGGTCTGAC
wSSIIA CGTCGTCCGG CTCAAATtTc GTGgTCTCGG CTTCTGCTCC CAGGCTGGAC
851
wSSIIB actgtCaGCG acGtGGaact TgaActGAAG aAGGgtgCgg tCattgTcaa 900
wSSIID ACTGTCAGCG ACGTGAACA AGAACTGAAG AAGGTGCGG TCGTTGTCGA
wSSIIA ATTGACAGCG ATGTTGAACC TGAActGAAG AAGGTGCGG TCATCGTCGA
901
wSSIIB aGAAGcTcCa aaCcCaAaAG CTCtTTcGCC GCCCGCAGCA CCCGCTGTAC 950
wSSIID AGAAGCTCCA AAGCCAAAGG CTCtTTcGCC GCctGCAGCc CCCGCTGTAC
wSSIIA AGAAGCTCCA AACCCAAAGG CTCtTTcGCC GCCTGCAGCC CCCGCTGTAC
951
wSSIIB AACAAAGACCT TTGGGACTTC AAGAAATACA TTGGTTTCGA GGAGCCCCGTG 1000
wSSIID AAgAAGACCT TTGGGAtTTC AAGAAATACA TTGGTTTCGA GGAGCCCCGTG
wSSIIA AAGAAGACCT TTGGGACTTC AAGAAATACA TTGGCTTCGA GGAGCCCCGTG

FIGURE 2E

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wSSIIIB	1001	GAGGCCAAGG	ATGATGGCCG	GGCTGTTGCA	GATGATGCGG	GCTCCTTCGA	1050
wSSIID		GAGGCCAAGG	ATGATGGCCG	GGCTGTcGCA	GATGATGCGG	GCTCCTTtGA	
wSSIIA		GAGGCCAAGG	ATGATGGCTG	GGCTGTTGCA	GATGATGCGG	GCTCCTTTGA	
wSSIIIB	1051	ACACCAACCAG	AATCACGATT	CCGGGCCCTTT	GGCAGGGGAG	AACGTCATGA	1100
wSSIID		ACACCAACCAG	AATCACGACT	CCGGaCCCTTT	GGCAGGGGAG	AAtGTCATGA	
wSSIIA		ACATCACCAAG	AACCATGATT	CCGGACCTTT	GGCAGGGGAG	AACGTCATGA	
wSSIIIB	1101	ACGTGGTCGT	CGTGGCTGCT	GAATGTTCTC	CCTGGTGCAA	AACAGGTGGT	1150
wSSIID		ACGTGGTCGT	CGTGGCTGCT	GAgTGTtCTC	CCTGGTGCAA	AACAGGTGGT	
wSSIIA		ACGTGGTCGT	CGTGGCTGCT	GAATGTTCTC	CCTGGTGCAA	AACAGGTGGT	
wSSIIIB	1151	CTTGGAGATG	TTGCCGGTGC	TTTGCCCAAG	GCTTTGGCGA	AGAGAGGACA	1200
wSSIID		CTgGGAGATG	TTGCgGGTGC	TcTGCCCAAG	GCTTTGGCaA	AGAGAGGACA	
wSSIIA		CTTGGAGATG	TTGCCGGTGC	TTTGCCCAAG	GCTTTGGCGA	AGAGAGGACA	

FIGURE 2F

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1201	1250				
wSSII B	TCGTGTTATG	GTTGTGGTAC	CAAGGTATGG	GGACTATGAG	GAAGCCTACG
wSSII D	TCGTGTTATG	GTTGTGGTAC	CAAGGTATGG	GGACTATGaa	GAACCTACGg
wSSII A	TCGTGTTATG	GTTGTGGTAC	CAAGGTATGG	GGACTATGAG	GAAGCCTACG
1251	1300				
wSSII B	ATGTCGGAGT	CCGAAAATAC	TACAAGGCTG	CTGGACAGGA	TATGGAAGTG
wSSII D	ATGTCGGAGT	CCGAAAATAC	TACAAGGCTG	CTGGACAGGA	TATGGAAGTG
wSSII A	ATGTCGGAGT	CCGAAAATAC	TACAAGGCTG	CTGGACAGGA	TATGGAAGTG
1301	1350				
wSSII B	AATTATTTC	ATGCTTATAT	CGATGGAGTT	GATTTGTGT	TCATTGACGC
wSSII D	AATTATTTC	ATGCTTaTAT	CGATGGAGTT	GATTTGTGT	TCATTGACGC
wSSII A	AATTATTTC	ATGCTTATAT	CGATGGAGTT	GATTTGTGT	TCATTGACGC
1351	1400				
wSSII B	TCCTCTCTTC	CGACACCGCC	AGGAAGACAT	TTATGGGGGC	AGCAGACAGG
wSSII D	TCCTCTCTTC	CGACACCGGAG	AGGAAGACAT	TTATGGGGGC	AGCAGACAGG
wSSII A	TCCTCTCTTC	CGACACCGCC	AGGAAGACAT	TTATGGGGGC	AGCAGACAGG

FIGURE 2G

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1401	1450				
wSSIIB	AAATTATGAA	GCGCATGATT	TTGTTCTGCA	AGGCCGCTGT	CGAGGTTCCA
wSSIID	AAATTATGAA	GCGCATGATT	TTGTTCTGCA	AGGCCGCTGT	TGAGGTTCCA
wSSIIA	AAATTATGAA	GCGCATGATT	TTGTTCTGCA	AGGCCGCTGT	CGAGGTTCCCT
1451	1500				
wSSIIB	TGGCACGTTT	CATGCGGCGG	TGTCCCTTAT	GGGATGGAA	ATCTGGTGTT
wSSIID	TGGCACGTTT	CATGCGGCGG	TGTCCCTTAT	GGGATGGAA	ATCTGGTGTT
wSSIIA	TGGCACGTTT	CATGCGGCGG	TGTCCCTTAT	GGGATGGAA	ATCTGGTGTT
1501	1550				
wSSIIB	TATTGCAAAT	GATTGGCACA	CGGCACTCCT	GCCTGTCTAT	CTGAAAGCAT
wSSIID	TATTGCAAAT	GATTGGCACA	CGGCACTCCT	GCCTGTCTAT	CTGAAAGCAT
wSSIIA	TATTGCAAAT	GATTGGCACA	CGGCACTCCT	GCCTGTCTAT	CTGAAAGCAT
1551	1600				
wSSIIB	ATTACAGGGA	CCATGGTTTG	ATGCAGTACA	CTCGGTCCAT	TATGGTGATA
wSSIID	ATTACAGGGA	CCATGGTTTG	ATGCAGTACA	CTCGGTCCAT	TATGGTGATA
wSSIIA	ATTACAGGGA	CCATGGTTTG	ATGCAGTACA	CTCGGTCCAT	TATGGTGATA

FIGURE 2H

1601	WSSIIB	CATAACATCG	CTCACCAGGG	CCGTGGCCCA	GTAGATGAGT	1650	TCCCGTTCAC
	WSSIID	CATAACATCG	CTCACCAGGG	CCGTGGCCCT	GTAGATGAAT		TCCCGTTCAC
	WSSIIA	CATAACATCG	CGCACCAGGG	CCGTGGCCCA	GTAGATGAAT		TCCCGTTCAC
1651	WSSIIB	CGAGTTGCCT	GAGCACTACC	TGGAACACTT	CAGACTGTAC	1700	GACCCCGTGG
	WSSIID	CGAGTTGCCT	GAGCACTACC	TGGAACACTT	CAGACTGTAC		GACCCCGTGG
	WSSIIA	CGAGTTGCCT	GAGCACTACC	TGGAACACTT	CAGACTGTAC		GACCCCGTGG
1701	WSSIIB	GTGGTGAACA	CGCCAACTAC	TTCGCCGCCG	GCCTGAAGAT	1750	GGCGGACCAG
	WSSIID	GTGGTGAACA	CGCCAACTAC	TTCGCCGCCG	GCCTGAAGAT		GGCGGACCAG
	WSSIIA	GTGGTGAACA	CGCCAACTAC	TTCGCCGCCG	GCCTGAAGAT		GgCGGACCAG
1751	WSSIIB	GTGTCGTCG	TGAGCCCCGG	GTACCTGTGG	GAGCTGAAGA	1800	CGGTGGAGGG
	WSSIID	GTGTCGTCG	TGAGCCCCGG	GTACCTGTGG	GAGCTGAAGA		CGGTGGAGGG
	WSSIIA	GTGTCGTCG	TGAGCCCCGG	GTACCTGTGG	GAGCTCAAGA		CGGTGGAGGG

FIGURE 2I

1801
wSSIIB CCGCTGGGG CTTACGACA TCATACGGCA GAACGACTGG AAGACCCGCG 1850
wSSIID CCGCTGGGG CTTACGACA TCATACGGCA GAACGACTGG AAGACCCGCG
wSSIIA CCGCTGGGG CTTACGACA TCATACGGCA GAACGACTGG AAGACCCGCG

1851
wSSIIB GCATCGTGAA CGGCATCGAC AACATGGAGT GGAACCCCGA GGTGGACGTC 1900
wSSIID GCATCGTCAA CGGCATCGAC AACATGGAGT GGAACCCCGA GGTGGACGCC
wSSIIA GCATCGTCAA CGGCATCGAC AACATGGAGT GGAACCCCGA GGTGGACGTC

1901
wSSIIB CACCTCAAGT CGGACGGCTA CACCAACTTC TCCCTGGGGA CGCTGGACTC 1950
wSSIID CACCTCAAGT CGGACGGCTA CACCAACTTC TCCCTGGGGA CGCTGGACTC
wSSIIA CACCTCAAGT CGGACGGCTA CACCAACTTC TCCCTGGGGA CGCTGGACTC

1951
wSSIIB CGGCAAGCGG CAGTGCAAGG AGGCCCTGCA GCGGAGAGTG GGCCTGCAGG 2000
wSSIID CGGCAAGCGG CAGTGCAAGG AGGCCCTGCA GCGGAGAGTG GGCCTGCAGG
wSSIIA CGGCAAGCGG CAGTGCAAGG AGGCCCTGCA GCGGAGAGTG GGCCTGCAGG

FIGURE 2J

Title: Genes Encoding Wheat Starch Synthases
and Uses Therefor

Inventors: Morrell et al.

Filing Date: October 29, 2001

WO 00/66745

T/AU00/00385

2001	2050
wSSIIB	TCGCGGGCGA CGTGCCGCTG CTCGGCTTCA TCGGGCGCCT GGACGGGCAG
wSSIID	TCGCGGGCGA CGTGCCGCTG CTCGGCTTCA TCGGGCGCCT GGACGGGCAG
wSSIIA	TCGCGGGCGA CGTGCCGCTG CTCGGCTTCA TCGGGCGCCT GGACGGGCAG
2051	2100
wSSIIB	AAGGGCGTGG AGATCATCGC GGACGCCGATG CCCTGGATCG TGAGCCAGGA
wSSIID	AAGGGCGTGG AGATCATCGC GGACGCCGATG CCCTGGATCG TGAGCCAGGA
wSSIIA	AAGGGCGTGG AGATCATCGC GGACGCCGATG CCCTGGATCG TGAGCCAGGA
2101	2150
wSSIIB	CGTGCAGCTG GTCATGCTGG GCACCGGGCG CCACGACCTG GAGGGCATGC
wSSIID	CGTGCAGCTG GTCATGCTGG GCACCGGGCG CCACGACCTG GAGGGCATGC
wSSIIA	CGTGCAGCTG GTCATGCTGG GCACCGGGCG CCACGACCTG GAGGGCATGC
2151	2200
wSSIIB	TGCGGCACTT CGAGCGGGAG CACCACGACA AGTGCGCGG GTGGGTGGGG
wSSIID	TGCGGCACTT CGAGCGGGAG CACCACGACA AGTGCGCGG GTGGGTGGGG
wSSIIA	TGCGGCACTT CGAGCGGGAG CACCACGACA AGTGCGCGG GTGGGTGGGG

FIGURE 2K

2201
wSSIIB TTCTCCGTGC GGCTGGCGCA CCGGATCACG GCCGGCGCCG ACGCGCTCCT 2250
wSSIID TTCTCCGTGC GCCTGGCGCA CCGGATCACG GCGGGGGCGG ACGCGCTCCT
wSSIIA TTCTCCGTgc GcCTGGCGCA CCGGATCACG GCGGGCGCCG ACGCGCTCct

2251
wSSIIB CATGCCCTCC CGGTCGAGC CGTGCGGACT GAACCCAGCTC TACGCCATGG 2300
wSSIID CATGCCCTCC CGGTCGTGC CGTGCGGGCT GAACCCAGCTC TACGCCATGG
wSSIIA CATGCCCTCC CGGTCGAgC CGTGCGGGTT GAACCCAGCTt TACGCCATGG

2301
wSSIIB CCTACGGCAC CGTCCCCGTC GTGCATGCCG TCGGTGGCCT GAGGGACACC 2350
wSSIID CCTACGGCAC CGTCCCCGTC GTGCACGCCG TCGCGGGCCT CAGGGACACC
wSSIIA CCTACGGCAC CGTCCCCGTC GTGCACGCCG TCGCGGGGT GAGGGACACC

2351
wSSIIB GTGCCGCCGT TCGACCCCTT CAACCACTCC GGGCTCGGGT GGACGTTCGA 2400
wSSIID GTGCCGCCGT TCGACCCCTT CAACCACTCC GGGCTCGGGT GGACGTTCGA
wSSIIA GTGCCGCCGT TCGACCCCTT CAACCACTCC GGCCTCGGGT GGACGTTCGA

FIGURE 2L

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2401      2450
wSSIIB    CCGCGCAGAG GCGCAGAAGC TGATCGAGGC GCTCGGGCAC TGCCTCCGCA
wSSIID    CCGCGCCCGAG GCGCACAAGC TGATCGAGGC GCTCGGGCAC TGCCTCCGCA
wSSIIA    CCGCGCCCGAG GCGCACAAGC TGATCGAGGC GCTCGGGCAC TGCCTCCGCA

2451      2500
wSSIIB    CCTACCGGGA CTACAAGGAG AGCTGGAGGG GGCTCCAGGA GCGCGGCATG
wSSIID    CCTACCGGAG CTTCAAGGAG AGCTGGAGGG CCCTCCAGGA GCGCGGCATG
wSSIIA    CCTACCGGGA CTACAAGGAG AGCTGGAGGG GGCTCCAGGA GCGCGGCATG

2501      2550
wSSIIB    TCGCAGGACT TCAGCTGGGA GCATGCCGCC AAGCTCTACG AGGACGTCCT
wSSIID    TCGCAGGACT TCAGCTGGGA GCATGCCGCC AAGCTCTACG AGGACGTCCT
wSSIIA    TCGCAGGACT TCAGCTGGGA GCATGCCGCC AAGCTCTACG AGGACGTCCT

2551      2600
wSSIIB    CGTCAAGGCC AAGTACCAGT GGTGAACGCT AGCTGCTAGC CGGTCCAGCC
wSSIID    CGTCAAGGCC AAGTACCAGT GGTGAACGCT AGCTGCTAGC CGGTCCAGCC
wSSIIA    CcTCAAGGCC AAGTACCAGT GGTGAACGCT AGCTGCTAGC CGcTCCAGCC
```

FIGURE 2M

Title: Genes Encoding Wheat Starch Synthases
and Uses Therefor

Inventors: Morrell et al.

Filing Date: October 29, 2001

WO 00/66745

U00/00385

```

2601      CCGCATGCG.    ...TGCATGA CAGGATGGAA TTGCGCATTG    2650
wSSIIB      CCGCATGCG.    ...TGCATGA CAGGATGGAA CT..GCATTG    CGCAGCGCAGG
wSSIID      CCGCATGCGT    GCATGcatga gAGGgTGGAa cTGCGCATTG    CGCAGCGCAGG
wSSIIA      CCGCATGCGT    GCATGcatga gAGGgTGGAa cTGCGCATTG    CGCccGCAGG

2651      AAGGTGCCAT    .....    .GGAGCGCCG    GCATCCGCCG    2700
wSSIIB      AAGGTGCCAT    .....    .GGAGCGCCG    GCATCCGCCG    AGTACAGTGA
wSSIID      AAcGTGCCAT    ccttctcgat    gGGAGCGCCG    GCATCCGCCG    AGTACAGTGA
wSSIIA      AAcGTGCCAT    ccttctcgat    gGGAGCGCCG    GCATCCGCCG    gGTgCAGTGA

2701      CAT..GAGGT    GTGTGTGGTT    GAGACGCTGA    TTC.....C    2750
wSSIIB      CAT..GAGGT    GTGTGTGGTT    GAGACGCTGA    TTC.....C    GATCTGGTCC
wSSIID      CATGAGagGT    GTGTGTGGTT    GAGACGCTGA    TTC.....C    AATCCGGCCC
wSSIIA      CATGAGagGT    GTGTGTGGTT    GAGACGCTGA    TTCGATCTc    gatctGGTCC

2751      GTAGCAGAGT    AGAGCGGAGG    TAGGGAAGCG    CTCCTTGTTA    2800
wSSIIB      GTAGCAGAGT    AGAGCGGAGG    TAGGGAAGCG    CTCCTTGTTA    CAGGTATATG
wSSIID      GTAGCAGAGT    AGAGCGGAGG    TAGGGAAGCG    CTCCTTGTTG    GTATTGTAAT
wSSIIA      GTAGCAGAGT    AGAGCGGAGG    TAGGGAAGCG    CTCCTTGTTg    CAGGTATATG
```

FIGURE 2N

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```
2801      2850
WSSIIB  GGAATGTTGT  TAACTTGGTA  TTGTAATTG  TTATGTTGTG  TGCATTATTA
WSSIID  TTGTTATGTT  GTGTGCATTA  TTACAATGTT  GTTACTTATT  CTTGTTAAGT
WSSIIA  GGAATGTTGT  CAACTTGGTA  TTGTAGTTG  CTATGTTGTa  TGCgTTATTA

2851      2900
WSSIIB  CAGAGGGCAA  CGATCTGCGC  CGGCGCACCG  GCCCAACTGT  TGGGCCGGTC
WSSIID  CGAGAGGCCAA  GGGCGAAAGC  TAGCTCACAT  GTCTGATGGA  TGCAAAAAAA
WSSIIA  caatgttgtt  acttattctt  gtTAAAAAAA  AAAAAAAA    AAAA~~~~~

2901      2950
WSSIIB  GCACAGCAGC  CGTTGGATCC  GACCGCCTGG  GCCGTTGGAT  CCCACCGAAA
WSSIID  AAAAAA      AAA~~~~~    ~~~~~~      ~~~~~~
WSSIIA  ~~~~~~      ~~~~~~      ~~~~~~      ~~~~~~

2951      2965
WSSIIB  AAAAAA      AAAAA
WSSIID  ~~~~~~      ~~~~~
WSSIIA  ~~~~~~      ~~~~~
```

FIGURE 20

FIGURE 3A

FIGURE 3B

FIGURE 3C

FIGURE 3D

FIGURE 3E

FIGURE 3F

FIGURE 3G

FIGURE 3

WSSIIA	1	MSSAVASAAS	---	FLALASA	SP-GRSRRRA	RVSAPPPHAG	AGRL----	HW	PPWPP-QRTA	51
WSSIIB	1	*****	---	*****	*****	***S***T*	*****	***	***S***	51
WSSIID		---	---	---	---	---	---	---	---	
ZSSIIA	1	***AV*SS*	STF*****	***G***	***GSS*F*T*	**S*SFAFWA	**S**RAPRD			57
ZSSIIB	1	*PG*-I*SS*	SAFL*PV**S	***-R***G	S*G*ALRSY*	YSGAELRL**	ARRG*P*DG*			56
PEASSII	1	*MLSLG*D*T	VLP*H*KNLK	FTP*KL*TLNG	--DLAFSKGL	GVGRINCGSV	-----R			49
POTSSII	10	PVNFIFCDFY	VMENSI*LHS	GNQFHPNLPL	---LALRPKK	LSLIHGSSRE	-----Q			57

↓ Transit peptide cleavage site

WSSIIA.	52	RDGGVAARAA	GKKDARVDDD	AASARQPRAR	RGGAATKVAE	RRDPVKTLDR	DAAEGGAPAP	111
WSSIIB	52	***A*****	***GI--**	**p*****L	*****	*****	*****S*	110
WSSIID		---	---	---	---	---	---	
ZSSIIA	58	AALVR*EAE*	*G***PPERS	GDA**L***	*---NA*SK	***	---	97
ZSSIIB	57	-ASVR**A*P	AGG-----	---	---	---	---	68
PEASSII	50	LNHKQHV**V	**SFGADENG	DG*EDDVVNA	TIEKSK**LA	LORELIQQIA	ERKKLVSSID	109
POTSSII	58	MWRNQVRVK*T	*ENSGEAA-S	*DESDALQV	TIEKSK**LA	MQQDLLQQIA	ERRKVVSSIK	116

FIGURE 3A

WSSIIA 112 PAPRQDAARP PSMNGTPVNG ENKSTGGGA TKDSGLPAPA RAPHSTQNR VPVNGENKAN 171
 WSSIIB 111 *****ED**L *****M***** *****Q**S*** ***** 170
 WSSIID -----
 ZSSIIA 98 -----LQPVG RYG*ATGNT* *TGAA*C**A ALADV*I*SI 132
 ZSSIIB 69 ----- -ESEEAAKSS SSSQAGAVQG STAKAVDS*S 97
 PEASSII 110 SDSIPGLEGN GVSYESSEKS LSR-----DS*P QKGSSSSGSA 146
 POTSSII 117 S-----SL*NA KGTYDGGSGS LSDVDIPDVD KDYNVTVPST A*TGITDVK NTPPAISHDF 172

 WSSIIA 172 VASPPTSIAE VVAPDSAATI SISDKAPESV VPAEKPPSS GSNEFVSASA PRLDIDSDVE 231
 WSSIIB 171 ***** **A***P***** *****A***** *****P***** *GS*TV***** 230
 WSSIID 203 ----- **A***VK FP**GYRMIL PSG*I**T* L**P*--LH E*PA*DGD*N --GIAPPT** 231
 ZSSIIA 134 ***** **A***VK FP**GYRMIL PSG*I**T* L**P*--LH E*PA*DGD*N --GIAPPT** 188
 ZSSIIB 99 PPN*L**APK QSQSAAMQNG TSGGSSASTA A*VSG*KADH P*AP*TKREI DASAVKPEPA 158
 PEASSII 147 *ETKR--WHC FQQ-----LC RSKETETWA* SSVGINQGFED EIEKKND*VK ASSKLHFNEQ 199
 POTSSII 173 *E*KREIKRD LADERAPPLS RS*IT*SSQI SSTVSSK--R TL*VPPETPK SSQETLL**N 230

FIGURE 3B

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WSSIIA	232	PELKKGAVIV	EEAPNPKALS	PPAAPAVQED	LWDFKKYIGF	WSSIIP1 Region	291
WSSIIB	231	L*****	K*****	*****Q*	*****	EEPVEAKDDG WAVADDAGSF	290
WSSIID	232	Q*****V*	*****K*****	*****	*****	*****R*****	291
ZSSIIA	189	*-----	-----	---L**A	T*****	*****R*****	224
ZSSIIB	159	GDDARPVESI	-----	-----	-----*I	D**D*****S RVG*****	188
PEASSII	200	IKN*LYERPD	TKDIS--SSI	R-----	-----TSSL	A**D**A- A*p*T**AAS	242
POTSSII	231	SRKSLVD*PG	KKIQSYMPSL	R-----	-----*ESSAS	KFENFEGANE PSSKEV*NEA	277
						HVEQRNENLE GSS*EANEET	

WSSIIA.	292	EHQNH--S	GPLAGENVMN	VVVAAECSP	WCKTGGLGDV	Region 1	Region 2
WSSIIB	291	*****--*	*****	*****	*****	AGALPKALAK	RGHRVMVVVP
WSSIID	292	*****--*	*****	*****	*****	*****	*****
ZSSIIA	225	**YGDN*--*	*****	*****	*****	*****	*****
ZSSIIB	189	APYDRE*NEP	*****P*****	*****S**A*	F*****	V*****	*****
PEASSII	243	*NFESGGEKP	P*****T*****	IIL*S**A*	*S*****	V*****	*****I*
POTSSII	278	*DPV*I*EKP	P*****T*****	IIL*S**A*	*S*****	*****	*****I*A*
						*****	*****A*

FIGURE 3C

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Sgp-1 Peptide 3

WSSIIA	350	RYGDYEEAYD	VGVRKYYKAA	GQDMEVNYEH	AYIDGVDFVF	IDAPLFRHRQ	EDIYGSRQE	409
WSSIIB	349	*****	*****	*****	*****	*****	*****	408
WSSIID	350	*****PT*	*****	*****	*****	*****E	*****	409
ZSSIIA	283	*****V**F*	*****	*****	*****	*****	*****	342
ZSSIIB	249	*****E**A**R*	L**RR**V*	*****S**T**	S*****	VE**P**H	NN**E*LD	308
PEASSII	303	H**N**A**H*	I**R**V*	*****T**	T*****I**	**S*I**NLE	SN**N*LD	362
POTSSII	338	*****DN**P**PQ*	S**I**VD	***VD**T**Q	*LLMDC*****	*HSHM**IG	NN**N*VD	397

Region 3

WSSIIA	410	IMKRMILFCK	AAVEPWHVP	CGGVPYGDGN	LVFIANDWHT	ALLPVYLKAY	YRDHGLMQYT	469
WSSIIB	409	*****	*****	*****	*****	*****	*****	468
WSSIID	410	*****	*****	*****	*****	*****	*****	469
ZSSIIA	343	*****	V*****	***C*****	*****	*****	*****	402
ZSSIIB	309	*L*****	*****YA*	***TV*****	*****	***N*****A	*****	368
PEASSII	363	*LR**V*****	*****	***IC*****	*****	*****N**	*****	422
POTSSII	398	*L***V*****	**I*****	***C*****	*****	***A*****	***N*I*N**	457

FIGURE 3D

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WSSIIA 470 RSIMVIHNIA HQGRGPVDEF PFTPEHYL EHERLYDPVG GEHANYFAAG LKMADQVVVV 529
WSSIIB 469 *****
WSSIID 470 *****
ZSSIIA 404 **VL***** Q**E*****I***** **T**R**T* 462
ZSSIIB 369 **VL***** D**K**NI* D*S*V***** **T**R**T* 428
PEASSII 423 **VL***** NTVD*SGN** DL*KM***** **F*I***** **T**R**T* 482
POTSSII 458 **VL***** SYVD**P**M DP*K***** **F*I***** **T**R**T* 517

Region 4

WSSIIA 530 SPGYLWELKT VEGGWGLHDI IRQNDWKTRG IVNGIDNMEW NPEVDVHLK- SDGYTNFSLG 588
WSSIIB 529 *****
WSSIID 530 *****
ZSSIIA 463 *R**M***** **S***IN* *****HQ** **K*****R- *****Y**E 521
ZSSIIB 429 *N**M***** *N*****LQ* *****MS** **A*****H- *****YTFE 487
PEASSII 483 *H**A***** *NES*****F** *****V*TKD* **QF*AY*T- *****YN*K 541
POTSSII 518 *H**S***** *NE*****LQ* *****TK** ***L*****PR *****M**Y**D 577

FIGURE 3E

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Region 5				Region 5a				
WSSIIA	589	TLDGKRQCK	EALQRELGLQ	VRADVPLLG	IGRLDGQKGV	EIIADAMPWI	VSQDVQLVML	648
WSSIIB	588	*****	*****	**G*****	*****	*****	*****	647
WSSIID	588	*****	*****	*****	*****	*****	*****	648
ZSSIIA	522	**A*****	A*****E	**D*****	*****	D**G*****	AG*****	581
ZSSIIB	488	**T*****	A***Q***	**D***I**	*****H*****	D*****IH**	AG*****	547
PEASSII	542	**QT*****	A*****P	**E***IIS*	*****H*****	DL**E*I**M	M*H*****	601
POTSSII	578	**QT**P**	A**K***P	**D***I**	*****P*****	DL**E*V**M	MG*****	637
Region 6				Region 6				
WSSIIA	649	GTGRHDLESM	LRHFEREHD	KVRGWVGFSV	RLAHRITAGA	DALLMPSRFE	PCGLNQLYAM	708
WSSIIB	648	*****G*	*****	*****	*****	*****	*****	707
WSSIID	649	*****	*Q*****	*****	*****	*****V	*****	708
ZSSIIA	582	***A***R*	*Q*L***PN	*****	PM*****	*V*****	*****	641
ZSSIIB	548	***A***D*	**R**S**S*	**A*****	P*****	*I*****	*****	607
PEASSII	602	***A***Q*	*KE**AQ*C*	*I*S*****	KM*****	*I*****	*****	661
POTSSII	638	***R***Q*	**Q**CQ*N*	*I*****	KTS*****	*I*****	**A*****	697

FIGURE 3F

WSSIIA	709	<u>AYGTPVVHA</u>	<u>VGGVRDTPPP</u>	<u>FDPFNHSGLG</u>	<u>WTFDRAEAKH</u>	<u>LIEALGHCLR</u>	<u>TYRDYKESWR</u>	768
WSSIIB	708	<u>*****</u>	<u>***L***</u>	<u>*****</u>	<u>*****Q*</u>	<u>*****</u>	<u>*****</u>	767
WSSIID	709	<u>*****</u>	<u>***L***</u>	<u>*****</u>	<u>*****</u>	<u>*****</u>	<u>***F***</u>	768
ZSSIIA	642	<u>*****</u>	<u>***L***A</u>	<u>***GDA***</u>	<u>*****N*</u>	<u>*****R***D</u>	<u>***K*G***K</u>	701
ZSSIIB	608	<u>*****</u>	<u>***L***A</u>	<u>***DT***</u>	<u>*****NR</u>	<u>M*D**S***T</u>	<u>***N***</u>	667
PEASSII	662	<u>S*****G</u>	<u>***L***Q*</u>	<u>*N**DE**V*</u>	<u>*****N*</u>	<u>*MA**WN**L</u>	<u>***K***K**E</u>	721
POTSSII	698	<u>K***I****</u>	<u>***L***Q*</u>	<u>**LMSQDW*</u>	<u>GPS*****SQ</u>	<u>**PRIRN**L</u>	<u>***E**K**E</u>	757

WSSIIA	769	GLQERMSQD	FSWEHAAKLY	EDVLLKAKYQ	W	799
WSSIIB	768	*****	*****	***V*****	*	798
WSSIID	769	*****	*****	***V*****	*	799
ZSSIIA	702	S**A*****	L**D***E**	***V*****	*	732
ZSSIIB	668	ACRA**AE*	L**D***V**	***V*****	*	698
PEASSII	722	*I*****	L**DN**QQ*	*E**VA*****	*	752
POTSSII	759	*I*T*C*T**	L**DN**QN*	*E**IA*****	*	788

FIGURE 3G

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anthesis



FIGURE 4

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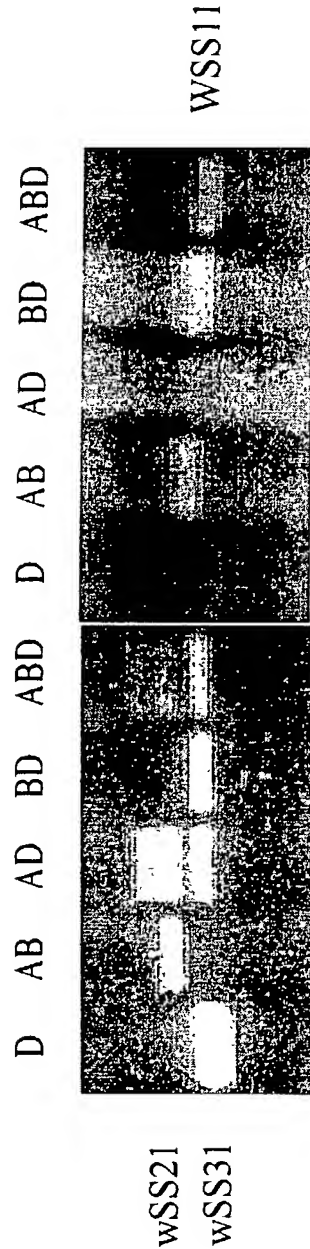


FIGURE 5

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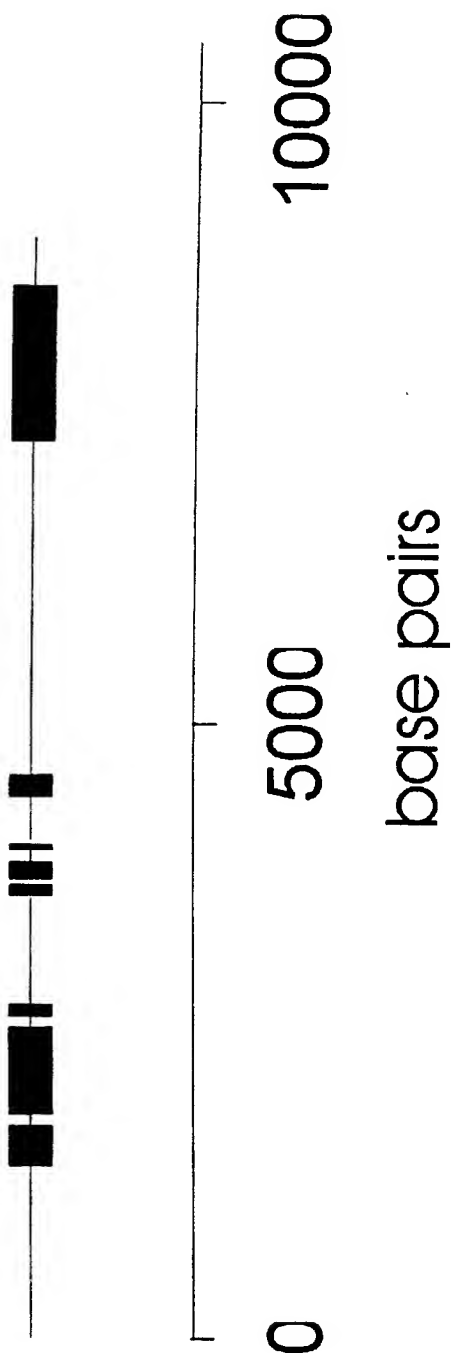


FIGURE 6

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3001841

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FIGURE 7A
FIGURE 7B
FIGURE 7C
FIGURE 7D
FIGURE 7E
FIGURE 7F
FIGURE 7G
FIGURE 7H
FIGURE 7I

FIGURE 7

1 MEMSLWPRSP LCPRSRQPLV VVRP..AGRG GLTQPFLLMG RFTRSRTRLRC 50
MEMVLRSQSP LCLRS.GPVL IFRPTVAGGG GGTQSLRRT RFARRRVIRC
pSSIII ~~~~~
51 MVASSDPPNR KSRMVPPQV KVISSRGYTT RLIVEPSNEN TEHNNRD... 100
VVASPGCPNR KS.RTASPNV KVAAYSNYAP RLLVESSSKK SEHHDSSRHR
pSSIII ~~~~~
101 EETLDTYNAL LSTETAETD NREAE..... ..TAKADSSQ NALSSSIIGE 150
EETIDTYNGL SGSDAAELTS NRDVEIEVDL QHISEEELPG KVSINASLGE
pSSIII ~~~~~
151 VDVAD..... EDILAADLTV YSLSSVMKKE VDAADKARVK EDAFELDLP 200
METVDEAEVE EDKFEVDTSI IVLNRNAVRE VDPKDEHNAK .DVFVVDSSG
pSSIII ~~~~~

FIGURE 7A

201	250	
wSSIII	TLRSVIVDVM	TLRSVIVDVM
mSSIII	TAPDNAAVEE	MVDVDILGLD
pSSIII	MDHNGTVQET	LRSVIVDVMD
	VVDEAEVEED	MVDVDILGLD
	VEVG..AVDE	AGSIKDRFET
	SGNVFSSSTT	VEVG..AVDE
	PEEGISIVHF	PEPNNDIVGS
	SGNVFSSSTT	VEVG..AVDE
	PEEGISIVHF	PEPNNDIVGS
	DAVDEVGPVQ	DKFEATSSGN
	QEQEQIVLSI	VDEEGLIASS
	VSNSATVREV	DASDE..AG
	CEEQGPVVDY	DKQEEENSTAF
	NDQGI FRADL	DEQKQLTDDF
	251	300
wSSIII	D.DAADKARV	GNISSAT..
mSSIII	DVDSPGNASS	GRTYGGVDEL
pSSIII	EEDVFELDL	GNISSAT..
	GRTYGGVDEL	GELPSTSVDC
	VSNSATVREV	DASDE..AG
	CEEQGPVVDY	DKQEEENSTAF
	NDQGI FRADL	DEQKQLTDDF
	301	350
wSSIII	DAVDEVGPVQ	DKFEATSSGN
mSSIII	QEQEQIVLSI	VDEEGLIASS
pSSIII	VSNSATVREV	DASDE..AG
	CEEQGPVVDY	DKQEEENSTAF
	NDQGI FRADL	DEQKQLTDDF
	351	400
wSSIII	SGNVFSSSTT	VEVG..AVDE
mSSIII	PEEGISIVHF	PEPNNDIVGS
pSSIII	SGNVFSSSTT	VEVG..AVDE
	PEEGISIVHF	PEPNNDIVGS
	PMWDAIDETV	TGLHEQDQSV

FIGURE 7B

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401      450
wSSIII  ADQDTFEADL  SGNASSCATY  REVDDVVDET  RSEETTFAMD  LFASESGHEK
mSSIII  VSSHGQDKSI  VG.VPQQIQY  NDQSIAGSHR  QDQSIAGAPE  QIQSVAGYIK
pSSIII  ~~~~~~  ~~~~~~  ~~~~~~  ~~~~~~  ~~~~~~MDVPF

451      500
wSSIII  HMAVDYVGEA  TDEEETYQQQ  YPVPSSFMSW  DKAIKTGVS  LNPELRLVRV
mSSIII  PNQ.SIVGSC  KQHELIPEP  KKIESIISYN  EIDQSIVGSH  KQDKSVVSV
pSSIII  PLHRSLSCTS  VSNAITHLKI  KPILGFVSHG  TTSLSVQSSS  WRKDGMMVTGV

501      550
wSSIII  EEQGVNFSD  KKDLSIDDL  GQNQSIIGSY  KQDKSIADVA  GPTQSI FGSS
mSSIII  EQIQSIVSHS  KPNQSTVDSY  RQAESIIGVP  EKVSITSYD  KLDQSI VGS
pSSIII  SFSICANFSG  RRRRKVSTPR  SQGSSPKGFV  PRKPSGMSTQ  RKVQKSNGDK

551      600
wSSIII  KQHR SIVAFP  KQNQSI VSVT  EQKQSI VGR  SQDLSAVSL.  .....P
mSSIII  KQDEPIISVP  EKIQSI VHYT  KPNQSI VGLP  KQQQSI VHIV  EPKQSI DGF
pSSIII  ESKSTSTSKE  SEISNQKTVE  ARVETSDDDT  KGVVRDHKFL  EDEDEINGST

```

FIGURE 7C

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601	650
wSSIII KQ.NVPIVGT SREGQTKQVP VVDRQDALYV NGLEAKEGDH TSEKTD DAL	
mSSIII KQ.DLSIVGI SNEFQTKQLA TVGTHDGLLM KGVEAKE... TSQKTEGDTL	
pSSIII KSISMSPPRV SSQFVESEET GGDDKDAVKL N..KSKRSEE SGFIIDSVIR	
651	700
wSSIII HVKFNVNDNL RKHQADRTQA VEKKTWKKVD EEHLYMTEHQ KRAA..EGQM	
mSSIII QATFNVDNLS QKQEGLTKEA DEITIIIEKIN DEDLVMIEEQ KSIAMNEEQ	
pSSIII EQSGSQGETN ASSKGSHAVG TKLYEILQVD VEPQQLEN. .NAGNVEYKG	
701	750
wSSIII VVNEDELSIT EIGMGRGD.K IQHVLSEEL SWSEDEVQLI EDDGQYEVDE	
mSSIII IVTEEDIPMA KVEIGIDKAK FLHLLSEES SWDENEVGII EADEQYEVDE	
pSSIII PVASKLLEIT KA.....SD VEHTESNEID DLDN..SFF KSDLIEEDEP	
751	800
wSSIII TSVSVNVEQD IQGSPQDVVD PQALKVMLQE LAEKNYSMRN KLFVFFPEVVK	
mSSIII TSMS..TEQD IQESPNDLD PQALWSMLQE LAEKNYSLGN KLFTYDPDLK	
pSSIII LAAGTVETGD SSLNLRLEME ANLRRQAIER LAEENLLQGI RLFCFPEVVK	

FIGURE 7D

801	ADSVIDLVLN	RDLTALANEP	DVVIKGAENG	WKWRLFTERL	HKSDLGGVWW	850
wSSIII	ADSTIDLVEN	RDLsAVANEP	DVLIKGAENG	WKWRFFTEKL	HKSELAGDWW	
mSSIII	PDEDVEIFLN	RGLSTLKNES	DVLIMGAENE	WRYRSFTTRL	TETHINGDWW	
pSSIII						
851	SCKLYIPKEA	YRLDFVFFNG	RTVYENNGNN	DFCIGIEGTM	NEDLFEDFLV	900
wSSIII	CCKLYIPKQA	YRMDFVFFNG	HTVYENNNNN	DFVIQIESTM	DENLFEDFLA	
mSSIII	SCKIHVPKEA	YRADFVFFNG	QDVYDNNDGN	DFSITVKGGM	QIIDFENFLL	
pSSIII						
901	KEKQRELEKL	AMEEAERRTQ	TEEQRRRKEA	RAADEAVRAQ	AKAEIEIKKK	950
wSSIII	EEKQRELENL	ANEEAERRRQ	TDEQRRMEEE	RAADKADRVQ	AKVEVETKKN	
mSSIII	EEKWREQEKL	AKEQAEERERL	AEEQRRIEAE	KAEIEADRAQ	AKEEAACKKK	
pSSIII						
951	KLQSMLSLAR	TCVDNLWYIE	ASTDTRGDTI	RLYYNRNSRP	LAHSTEIWMH	1000
wSSIII	KLCNVLGLAR	APVDNLWYIE	PITGQEATV	RLYYNINSRP	L VHSTEIWMH	
mSSIII	VLRELMVKAT	KTRDITWYIE	PSEFKCEDKV	RLYYNKSSGP	LSHAKDLWIH	
pSSIII						

FIGURE 7E

1001
 wSSIII GGYNNWTDGL SIVESFVKCN DKDGDWWYAD VIPPEKALVL DWVFADGPAG 1050
 mSSIII GGYNNWIDGL SFAERLVHHH DKDCDWWFAD VVVPERTYVL DWVFADGPPG
 pSSIII GGYNNWKDGL SIVKKLVKSE RIDGDWWYTE VVIPDQALFL DWVFADGPPK

 1051
 wSSIII NARNYDNNAR QDFHAILPNN NVTEEGFWAQ EEQNIYTRLL QERREKEETM 1100
 mSSIII SARNYDNNGG HDEFHATLP.N NMTEEEYWME EEQRIYTRLQ QERREREAI
 pSSIII HAIAYDNNHR QDFHAIVP.N HIPEELYWVE EEHQIFKTLQ EERRLEAAM

 1101
 wSSIII KRKAERSANI KAEMKAKTMR RFLSQKHIV YTEPLEIRAG TTVDVLYNPS 1150
 mSSIII KRKAERNAKM KAEMKEKTMR MFLVSQKHIV YTEPLEIHAG TTIDVLYNPS
 pSSIII RAKVEKTALL KTETKERTMK SFLLSQKHVV YTEPLDIQAG SSVTVYYNPA

 1151
 wSSIII NTVLNGKSEG WFRCSFNLWM HSSGALPPQK MVKSGDGP LL KATVDVPPDA 1200
 mSSIII NTVLTGKPEV WFRCSFNRWM YPGGVLPQK MVQAENGSHL KATVYVPRDA
 pSSIII NTVLNGKPEI WFRCSFNRWT HRLGPLPPQK MSPAENGTHV RATVKVPLDA

FIGURE 7F

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1201	wSSIII	YMMDFVFW	EEDGIYDNRN	GMDYHIPVSD	SIETENYMRI	IHIAVEMAPV	1250
	mSSIII	YMMDFVFW	EEDGIYDNRN	GMDYHIPVFG	SIKEPPMHI	VHIAVEMAPI	
	pSSIII	YMMDFVFW	EEDGIYDNRN	GMDYHIPVFG	GVAKEPPMHI	VHIAVEMAPI	
1251	wSSIII	AKVGGGLGDVV	TSLSRAIQDL	GHTVEVILPK	YDCLNQSSVK	DLHLYQSFWS	1300
	mSSIII	AKVGGGLGDVV	TSLSRAVQDL	GHNVEVILPK	YGCLNLSNVK	NLQIHQSFWS	
	pSSIII	AKVGGGLGDVV	TSLSRAVQDL	NHNVDIILPK	YDCLKMNNVK	DFRFHKNYFW	
1301	wSSIII	GGTEIKVWVG	RVEDLTVYFL	EPQNGMFGVG	CVYG.RNDDR	RFGFFCHSAL	1350
	mSSIII	GGSEINWVRG	LVEGLCVYFL	EPQNGMFGVG	YVYG.RDDDR	RFGFFCRSAL	
	pSSIII	GGTEIKVWVG	KVEGLSVYFL	EPQNGLFSKG	CVYGCSNDGE	RFGFFCHAAL	
1351	wSSIII	EFILQNEFSP	HIHCHDWSS	APVAWLKHE	YSQSRMASTR	VVFTIHNLEF	1400
	mSSIII	EFLQSGSSP	NIIHCHDWSS	APVAWLHKN	YAKSSLANAR	VVFTIHNLEF	
	pSSIII	EFLQGGFSP	DIIHCHDWSS	APVAWLFKEQ	YTHYGLSKSR	IVFTIHNLEF	

FIGURE 7G

1401	GAHYIGKAMT	YCDKATTVSP	TYSRDVAGHG	AIAPHREKFY	GILNGIDPDI	1450
wSSIII						
mSSIII	GAHHIGKAMR	YCDKATTVSN	TYSKEVSGHG	AIVPHLGKFY	GILNGIDPDI	
pSSIII	GADLIGRAMT	NADKATTVSP	TYSQEVSGNP	VIAPHLHKFH	GIVNGIDPDI	
1451	WDPYTDNFIP	VPYTCENVVE	GKRAAKRALQ	QKFGGLQQTDV	PIVGIIITRLT	1500
wSSIII						
mSSIII	WDPYNDNFIP	VHYTCENVVE	GKRAAKRALQ	QKFGGLQQIDV	PVVGIVITRLT	
pSSIII	WDPLNDKFIP	IPYTSENVVE	GKTAAKEALQ	RKLGLKQADL	PLVGIIITRLT	
1501	AQKGIHLIKH	AIHRTLESNG	HVVLLGSAPD	HRIQGDFCRL	ADALHGVYHG	1550
wSSIII						
mSSIII	AQKGIHLIKH	AIHRTLERNG	QVVLLGSAPD	SRIQADFVNL	ANTLHGVNHG	
pSSIII	HQKGIHLIKH	AIWRTLERNG	QVVLLGSAPD	PRVQNNFVNL	ANQLHSKYND	
1551	RVKLVLTIDE	PLSHLIYAGS	DFIIVPSIFE	PCGLTQLVAM	RYGSIPIVRK	1600
wSSIII						
mSSIII	QVRLSLTYDE	PLSHLIYAGS	DFILVPSIFE	PCGLTQLVAM	RYGTIPIVRK	
pSSIII	RARLCLTYDE	PLSHLIYAGA	DFILVPSIFE	PCGLTQLTAM	RYGSIPIVVRK	

FIGURE 7H

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1601	1650				
wSSIII	TGGLHDTVFD	VDNDKDRARS	LGLEPNGFSF	DGADSNQVDY	ALNRAIGAWF
mSSIII	TGGLFDTVFD	VDNDKERARD	RGLEPNGFSF	DGADSNQVDY	ALNRAISAWF
pSSIII	TGGLYDTVFD	VDHDKERAQQ	CGLEPNGFSF	DGADAGGVDY	ALNRALSAWY
1651	1689				
wSSIII	DARDWFHSLC	KRVMEQDWSW	NRPALDYIEL	YHAARKF*	
mSSIII	DARSWFHSLC	KRVMEQDWSW	NRPALDYIEL	YRSASKL~	
pSSIII	DGRDWFNSLC	KQVMEQDWSW	NRPALDYIEL	YHAARKLE*	

FIGURE 7I

Title: Genes Encoding Wheat Starch Synthases

and Uses Therefor

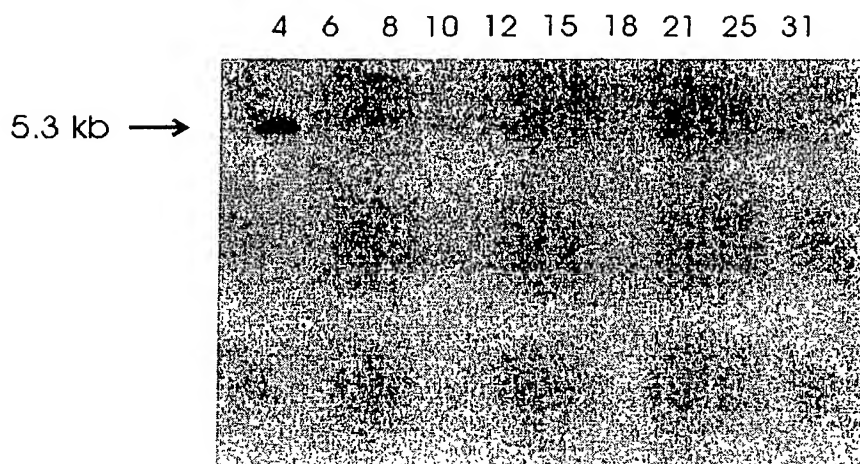
Inventors: Morrell et al.

Filing Date: October 29, 2001

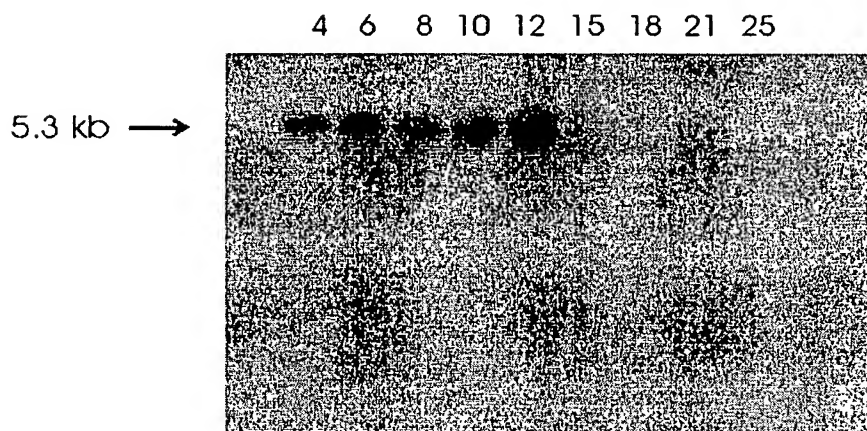
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/AU00/00385

[a] Wyuna



[b] Gabo



[c] Gabo



FIGURE 8

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FIGURE 9A	FIGURE 9B
FIGURE 9C	FIGURE 9D
FIGURE 9E	FIGURE 9F

FIGURE 9

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		Region 1			Region 2		
		20			40		
		10			50		
wGBSS	81	FVGAEMAPWS	KTGGLGDLG	GLPPAMAANG	HRVMVISPRY	DQYKDAWDT-	
wSS1	144	-*TG*A**YA	*S*****VC*	S**I*L**R*	*****VM***	LNGSSDKNYA	
wSS2	314	--A**CS**C	*****VA*	A**K*L*KR*	*****VV***	GD*EE*Y*V-	
wSS3	1187	-IAV*****VA	V*****VVT	S*SR*IQDL*	*T*E**L*K*	*CLNQSSVK-	
		100			130		
		110			140		
wGBSS	171	LEKVRGKTKE	KIYGPDA GTD	YEDNQQRFSL	LCQAALVPR	ILNLDNNPYF	
wSS1	234	-HRPGSLYGD	-----NFGA	FG***F*YT*	**Y**C*A*L	**E*GGYI*G	
wSS2	404	RHRQEDIYGG	-----S	RQEIMK*MI*	F*K**V***W	HVPCGGV**G	
wSS3	1277	*PQN*MFGV	-----GCVY	GRNDDR**GF	F*HS***--F	**QNEFS*H-	
		190			220		
		200			230		
wGBSS	261	FCIHNI SYQG	RESFD DFAQL	NLPD-----R	FKSSFDFIDG	YDKPVEGRKI	
wSS1	324	LV***LAH**	LEPASTYPD*	G**PEWYGAL	EWVFPEWARR	HALDKGEAVN	
wSS2	494	MV***AH**	*GPV*E*PFT	E*-----	-EHYLEHFRL	**PVGGEHAN	
wSS3	1367	*T***L-EF*	AHYIGKAMTY	CDK-----	-----	-----	

FIGURE 9A

60	70	80	90	
-----SVVSE	IKVVDKYERV	RYFHCYKRGV	DRVFVDHPCF	170
KALYTGKHIK	*PCFGGSHE*	TF**E*RDN*	*W*****SY	233
-----G*PKY	Y*AAQDME*	N***A*ID**	*F**I*A*L*	403
-----	-DLHLYQSFS	WGGTEI*VW*	G**EDLTVY*	1276
Region 3				
150	160	170	180	
SGPYGEDVVF	VCNDWHTGLL	ACYLKSNIQS	NGIYRAAKVA	260
QN-----CM*	*V***AS*V	PVL*AAK*RP	Y*V**DSRST	323
D*-----NL**	IA*****A**	PV***AY*RD	H*LMQYTRSI	493
-----II	H*H**SSAPV	*WLY*EH*SQ	-SRMASTR*V	1366
240	250	260	270	
NWMKAGILQA	DKVLTVSPYY	AEELISGEAR	GCELDNIMRL	350
FLKG*VVTAD	RI*TVSQG*S	W*VTTAEGGQ	*LNEILLSS*K	413
YFAAGLKMAD	QV*VVSPG*L	W*LKTVEGGW	*LHDIIRQND	583
-----	-----AT	TVSPTYSRDV	AGHGAIAPHR	1456

FIGURE 9B

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Region 4

	280	290	300	310	320
wGBSS	351	TGITTIVNGM DVSEWDPTKD	KFLAVNYDIT	TALEGKALNK	EALEGKALNK
wSS1	414	SVLNG***I *IND*N**T*	*C*PHH*SV-	-----	DD*S**KC*
wSS2	584	WKTRG***I *NM**N*EV*	VH*KSDGYTN	-----FSLG	TLDS**RQC*
wSS3	1457	EKFYGL**I *PDI***YT*	N*IP*P*TCE	-----NVVEG*	**AKRALQQ*

Region 5a

	370	380	390	400	410
wGBSS	441	LKEEDVQIVL LGTGKKKFER	LLKSIEEKFP	SKVRVVREN	-----APLA
wSS1	504	*MR***F*M **S*DPI**G	WMR*T*SSYK	D*F*GW*G*S	-----V*VS
wSS2	674	V-SQ***L*M ***RHDLS	M*RHF*REHH	D**GW*G*S	-----VR**
wSS3	1547	TL*SNG*V** **SAPDHRIQ	GDFCRLADAL	HG*YHGRVKL	-VLTIDE**S

FIGURE 9C

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Region 5			
330	340	350	360
EALQAEVGLP	VDRKVPLVAF	IGRLEEQKGP	DVMIASIP EI
AE**K*L**	*RED***IG*	****DY***I	*LIKMA***-
****R*L**Q	*RAD***LG*	****DG***V	EIIADAM*W*
FG**QT----	---D**I*GI	*T**TA***I	-HL*KHAIHR
			440 503 673 1546
Region 6		Region 7	
420	430	440	450
HQMMAGADVL	AVTSRFEPCG	LIQLQGMRYG	TPCACASTGG
*RIT**C*I*	LMP*****	*N**YA*Q**	*VPVVHG***
*RIT***A*	LMP*****	*N**YA*A**	*VPVVHAV**
*LIY**S*FI	I*P*I*****	*T**VA***	SIPIVRK***
			530 593 763 1636

FIGURE 9D

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Region 7 (Continued)

	460	470	480	490	500						
wGBSS 531	LVD	TIVEGKT	GFHMGR	LSYD	CNV	VEPADVK	KVV	TTLKRAV	KVV	GTPAYHE	
wSS1 594	*R	*--*	*TFN	---	--	PFGAKGEE	GTG	WAFSPLT	VDK	MLW*LRT	
wSS2 764	VR	*--*	*PPFD	---	--	PNHSGSLG	--	-W*FD**E	AHK	LIE*LGH	
wSS3 1637	**	*--*	*FDV	NDK	DRAR*LG	LEP	NGFSFDG	ADSN	GV	VDY*L	
										NRA	IGAWFDA
550	560	570	580	590	600						
wGBSS 621	APL	AMEN	VAA	P*							
wSS1 684	FVD	QPY	VM..								
wSS2 854	KYQ	W.....									
wSS3 1727											

FIGURE 9E

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11049418 : 104018418

510	MVKNCMIQDL	SWKGPAKNWE	DVLELGVEG	SEPGIVGEEI	620
	AMSTFEHKEP	**E*LM*RG	TKDHTWDHAA	EQYEQIF*WA	683
	CLRTYRDYKE	**R*LQERGM	SQDFSWEHAA	KLYED*LLKA	853
	RDWFHSLCKK	VMEQDWSWNR	PA*DYIELYH	AARKF*....	1726
610		620	630		
	710
	773
	943
	1816

FIGURE 9F

Title: Genes Encoding Wheat Starch Synthases
and Uses Therefor

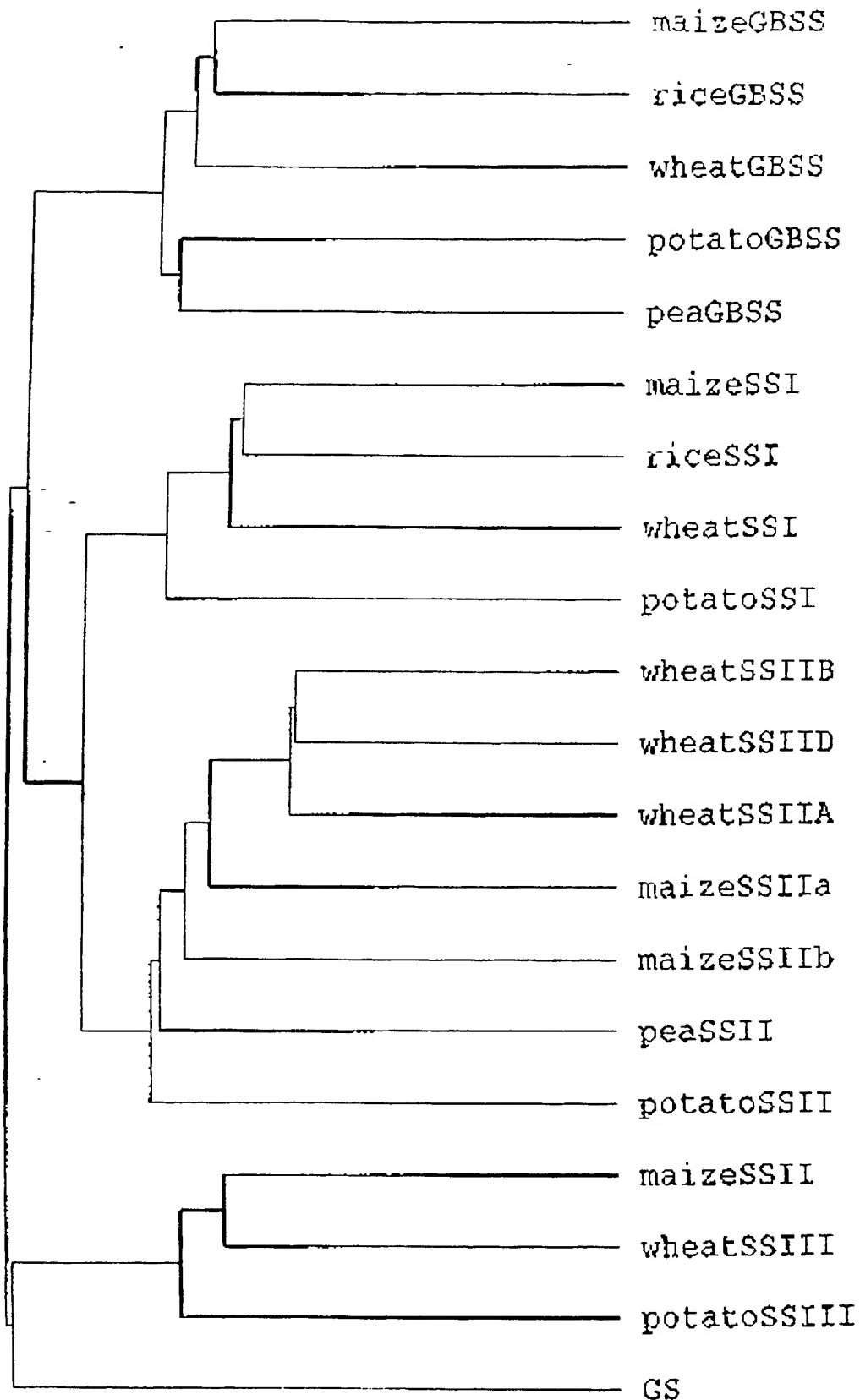
Inventors: Morrell et al.

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4/1/00

**FIGURE 10**

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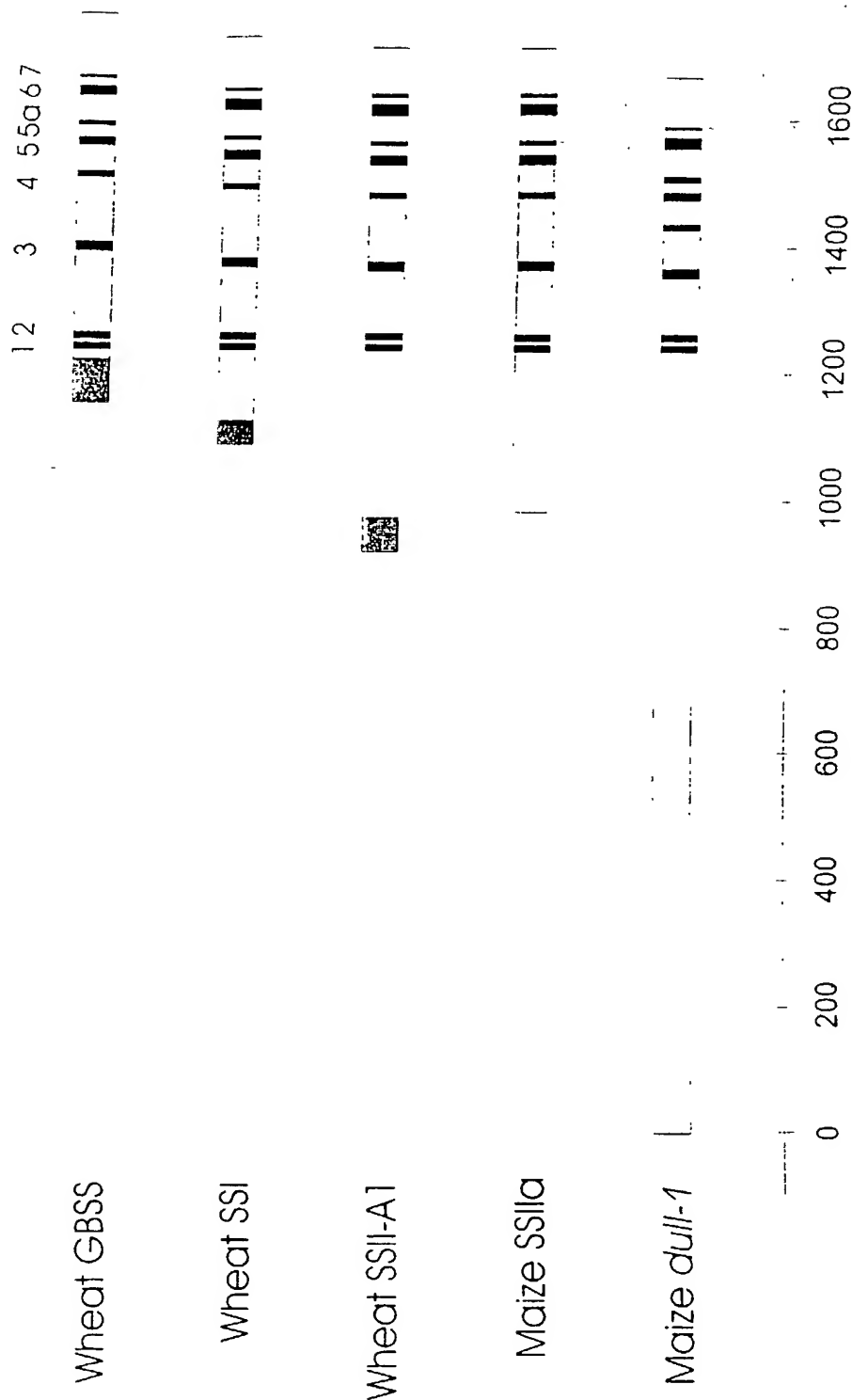


FIGURE 11

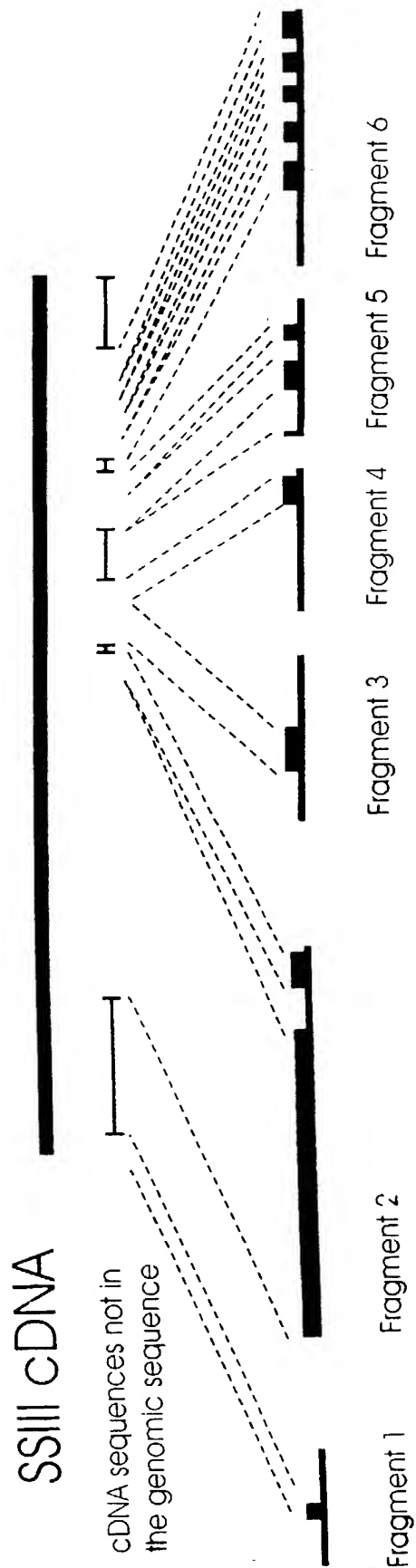


FIGURE 12

8901 11

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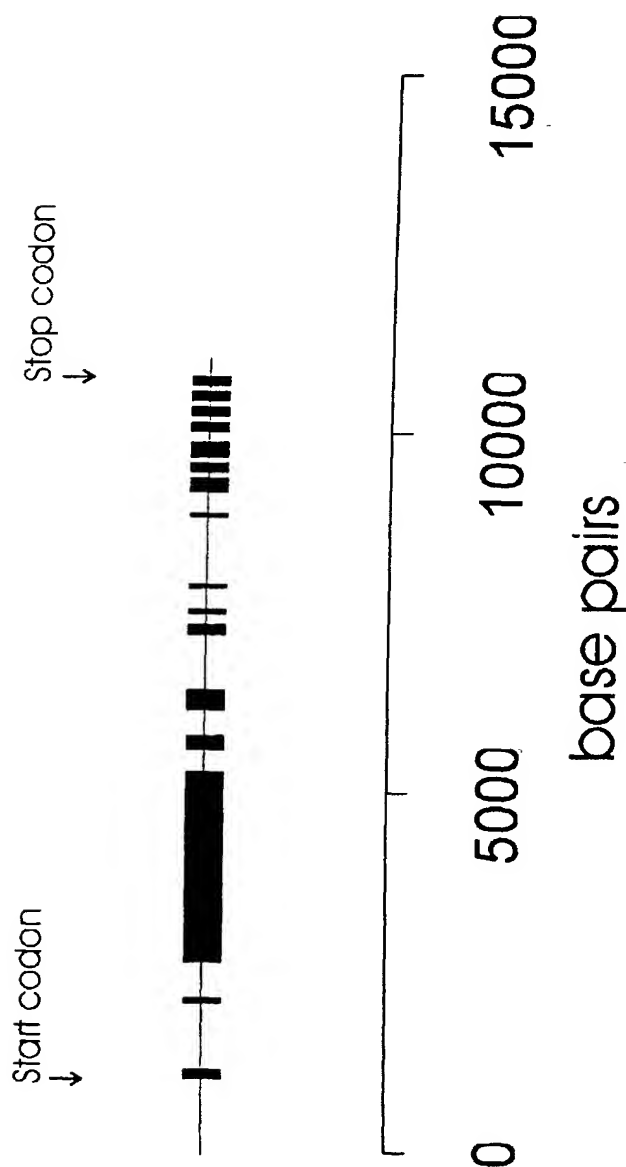
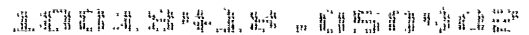


FIGURE 13



As the below named inventors, we hereby declare that:

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled:

the specification of which was filed on 28 April 2000 as PCT/AU00/00385 and was amended on 29 October, 2001.

We acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

We hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application to which priority is claimed:

Page 1 of 4

Prior Provisional Application(s)

We hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application Serial
Number

Date of Filing
(day,month,year)

Prior U.S. Application(s) and PCT International Application(s) Designating the United States

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT International application(s) designating the United States listed below:

Application Serial
Number

Date of Filing
(day,month,year)

Status(Patented,Pending,Abandoned)

Insofar as the subject matter of each of the claims in this application is not disclosed in the prior United States, foreign or PCT International application(s) to which priority has been claimed above in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

We hereby appoint, both jointly and severally, as our attorneys and agents with full power of substitution and revocation, to prosecute this application and any corresponding application filed in the Patent Cooperation Treaty Receiving Office, and to transact all business in the Patent and Trademark Office connected herewith the following attorneys and agents, their registration numbers being listed after their names:

(10) Lorance L. Greenlee, Reg. No. 27,894; Ellen P. Winner, Reg. No. 28,547; Sally A. Sullivan, Reg. No. 32,064; Donna M. Ferber, Reg. No. 33,878; G. William VanCleave, Reg. No. 40,213, Susan K. Doughty, Reg. No. 43,595, Heeja Yoo-Warren, Reg. No. 45,495, and Tamala R. Jonas, Reg. No. 47,688; Jonathan A. Baker, Reg. No. 49,022; and Mary Beth Vellequette, Reg. No. 47,903, all of Greenlee, Winner and Sullivan, P.C., 5370 Manhattan Circle, Suite 201, Boulder, CO 80303.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

(1) Full Name of First Inventor: 1-00 Matthew MORELL
Residence: Aranda, Australia
Citizenship: Australian AUX
Post Office Address: 33 Wangara Street
Aranda
Australian Capital Territory 2614
Australia

(1) Signature x Matthew Morell Date x 13/12/01

(2) Full Name of Second Inventor: 2-00 Zhongyi LI
Residence: Kaleen, Australia
Citizenship: Australian AUX
Post Office Address: 63 Campaspe Circuit
Kaleen
Australian Capital Territory 2617
Australia

(2) Signature x [Signature] Date x 7/12/01

(3) Full Name of Third Inventor: 3-00 Sadequr RAHMAN
Residence: Melba, Australia
Citizenship: Australian AUX
Post Office Address: 46 Scarlett Street
Melba
Australian Capital Territory 2615
Australia

(3) Signature x Sadequr Rahman Date x 10/1/02

(4) Full Name of Fourth Inventor: 400 Rudolph APPELS
Residence: Aranda, Australia
Citizenship: Australian AUX
Post Office Address: 40 Gingara Street 67c. Gardner St
Aranda Como, Perth, Western Australia
Australian Capital Territory 2614 6151
Australia

(4) Signature x [Signature] Date x 21/01/02

- 1 -

SEQUENCE LISTING

<110> COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
 GOODMAN FIELDER LIMITED
 GROUPE LIMAGRAIN PACIFIC PTY LTD

<120> NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES
 THEREFOR

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<140> TO BE ADVISED

<141> 2000-04-28

<150> AU PQ0052/99

<151> 1999-04-29

<160> 54

<170> PatentIn Ver. 2.0

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 Met
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 35 40 45

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 Thr Ala Arg Asp Gly Ala Val Ala Ala Arg Ala Ala Gly Lys Lys Asp
 50 55 60 65

gcg ggg atc gac gac gcc gcg ccc gcg agg cag ccc cgc gca ctc cgc 418
 Ala Gly Ile Asp Asp Ala Ala Pro Ala Arg Gln Pro Arg Ala Leu Arg
 70 75 80

ggt ggc gcc gcc acc aag gtt gcg gag ccg agg gat ccc gtc aag acg 466
 Gly Gly Ala Ala Thr Lys Val Ala Glu Arg Arg Asp Pro Val Lys Thr
 85 90 95

ctc gat cgc gac gcc gcg gaa ggt ggc gcg ccg tcc ccg ccg gca ccg 514
 Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ser Pro Pro Ala Pro

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100	105	110	
agg cag gag gac gcc cgt ctg ccg agc atg aac ggc atg ccg gtg aac			562
Arg Gln Glu Asp Ala Arg Leu Pro Ser Met Asn Gly Met Pro Val Asn			
115	120	125	
ggt gaa aac aaa tct acc ggc ggc ggc ggc gcg act aaa gac agc ggg			610
Gly Glu Asn Lys Ser Thr Gly Gly Gly Gly Ala Thr Lys Asp Ser Gly			
130	135	140	145
ctg ccc gca ccc gca cgc gcg ccc cag ccg tcg agc cag aac aga gta			658
Leu Pro Ala Pro Ala Arg Ala Pro Gln Pro Ser Ser Gln Asn Arg Val			
150	155	160	
ccg gtg aat ggt gaa aac aaa gct aac gtc gcc tcg ccg ccg acg agc			706
Pro Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro Thr Ser			
165	170	175	
ata gcc gag gtc gcg gct ccg gat ccc gca gct acc att tcc atc agt			754
Ile Ala Glu Val Ala Ala Pro Asp Pro Ala Ala Thr Ile Ser Ile Ser			
180	185	190	
gac aag gcg cca gag tcc gtt gtc cca gcc gag aag gcg ccg ccg tcg			802
Asp Lys Ala Pro Glu Ser Val Val Pro Ala Glu Lys Ala Pro Pro Ser			
195	200	205	
tcc ggc tca aat ttc gtg ccc tcg gct tct gct ccc ggg tct gac act			850
Ser Gly Ser Asn Phe Val Pro Ser Ala Ser Ala Pro Gly Ser Asp Thr			
210	215	220	225
gtc agc gac gtg gaa ctt gaa ctg aag aag ggt gcg gtc att gtc aaa			898
Val Ser Asp Val Glu Leu Glu Leu Lys Lys Gly Ala Val Ile Val Lys			
230	235	240	
gaa gct cca aac cca aag gct ctt tcg ccg ccc gca gca ccc gct gta			946
Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro Ala Val			
245	250	255	
caa caa gac ctt tgg gac ttc aag aaa tac att ggt ttc gag gag ccc			994
Gln Gln Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu Glu Pro			
260	265	270	
gtg gag gcc aag gat gat ggc cgg gct gtt gca gat gat gcg ggc tcc			1042
Val Glu Ala Lys Asp Asp Gly Arg Ala Val Ala Asp Asp Ala Gly Ser			
275	280	285	
ttc gaa cac cac cag aat cac gat tcc ggg cct ttg gca ggg gag aac			1090
Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly Glu Asn			
290	295	300	305
gtc atg aac gtg gtc gtc gtg gct gct gaa tgt tct ccc tgg tgc aaa			1138
Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp Cys Lys			
310	315	320	
aca ggt ggt ctt gga gat gtt gcc ggt gct ttg ccc aag gct ttg gcg			1186
Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala Leu Ala			
325	330	335	
aag aga gga cat cgt gtt atg gtt gtg gta cca agg tat ggg gac tat			1234
Lys Arg Gly His Arg Val Met Val Val Val Pro Arg Tyr Gly Asp Tyr			
340	345	350	
gag gaa gcc tac gat gtc gga gtc cga aaa tac tac aag gct gct gga			1282
Glu Glu Ala Tyr Asp Val gga gtc cga aaa tac Tyr Lys Ala Ala Gly			
355	360	365	

cag Gln 370	gat Asp	atg Met	gaa Glu	gtg Val	aat Asn	tat Tyr	ttc Phe	cat His	gct Ala	tat Tyr	atc Ile	gat Asp	gga Gly	gtt Val	gat Asp	1330
ttt Phe	gtg Val	ttc Phe	att Ile	gac Asp	gct Ala	cct Pro	ctc Leu	ttc Phe	cga Arg	cac His	cgc Arg	cag Gln	gaa Glu	gac Asp	att Ile	1378
tat Tyr	ggg Gly	ggc Gly	agc Ser	aga Arg	cag Gln	gaa Glu	att Ile	atg Met	aag Lys	cgc Arg	atg Met	att Ile	ttg Leu	ttc Phe	tgc Cys	1426
aag Lys	gcc Ala	gct Ala	gtc Val	gag Glu	gtt Val	cca Pro	tgg Trp	cac His	gtt Val	cca Pro	tgc Cys	ggc Gly	ggg Gly	gtc Val	cct Pro	1474
tat Tyr	ggg Gly	gat Asp	gga Gly	aat Asn	ctg Leu	gtg Val	ttt Phe	att Ile	gca Ala	aat Asn	gat Asp	tgg Trp	cac His	acg Thr	gca Ala	1522
ctc Leu	ctg Leu	cct Pro	gtc Val	tat Tyr	ctg Leu	aaa Lys	gca Ala	tat Tyr	tac Tyr	agg Arg	gac Asp	cat His	ggg Gly	ttg Leu	atg Met	1570
cag Gln	tac Tyr	act Thr	cgg Arg	tcc Ser	att Ile	atg Met	gtg Val	ata Ile	cat His	aac Asn	atc Ile	gct Ala	cac His	cag Gln	ggc Gly	1618
cgt Arg	ggc Gly	cca Pro	gta Val	gat Asp	gag Glu	ttc Phe	ccg Pro	ttc Phe	acc Thr	gag Glu	ttg Leu	cct Pro	gag Glu	cac His	tac Tyr	1666
ctg Leu	gaa Glu	cac His	ttc Phe	aga Arg	ctg Leu	tac Tyr	gac Asp	ccc Pro	gtg Val	ggg Gly	ggg Gly	gaa Glu	cac His	gcc Ala	aac Asn	1714
tac Tyr	ttc Phe	gcc Ala	gcc Ala	ggc Gly	ctg Leu	aag Lys	atg Met	gcg Ala	gac Asp	cag Gln	gtt Val	gtc Val	gtc Val	gtg Val	agc Ser	1762
ccg Pro	ggg Gly	tac Tyr	ctg Leu	tgg Trp	gag Glu	ctg Leu	aag Lys	acg Thr	gtg Val	gag Glu	ggc Gly	ggc Gly	tgg Trp	ggg Gly	ctt Leu	1810
cac His	gac Asp	atc Ile	ata Ile	cgg Arg	cag Gln	aac Asn	gac Asp	tgg Trp	aag Lys	acc Thr	cgc Arg	ggc Gly	atc Ile	gtg Val	aac Asn	1858
ggc Gly	atc Ile	gac Asp	aac Asn	atg Met	gag Glu	tgg Trp	aac Asn	ccc Pro	gag Glu	gtg Val	gac Asp	gtc Val	cac His	ctc Leu	aag Lys	1906
tcg Ser	gac Asp	ggc Gly	tac Tyr	acc Thr	aac Asn	ttc Phe	tcc Ser	ctg Leu	ggg Gly	acg Thr	ctg Leu	gac Asp	tcc Ser	ggc Gly	aag Lys	1954
cgg Arg	cag Gln	tgc Cys	aag Lys	gag Glu	gcc Ala	ctg Leu	cag Gln	cgg Arg	gag Glu	ctg Leu	ggc Gly	ctg Leu	cag Gln	gtc Val	cgc Arg	2002
ggc Gly	gac Asp	gtg Val	ccg Pro	ctg Leu	ctc Leu	ggc Gly	ttc Phe	atc Ile	ggg Gly	cgc Arg	ctg Leu	gac Asp	ggg Gly	cag Gln	aag Lys	2050

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- 4 -

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Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu Gly Met
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ctg cgg cac ttc gag cgg gag cac cac gac aag gtg cgc ggg tgg gtg 2194
Leu Arg His Phe Glu Arg Glu His His Asp Lys Val Arg Gly Trp Val
660 665 670

ggg ttc tcc gtg cgg ctg gcg cac cgg atc acg gcc ggc gcc gac gcg 2242
Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala Asp Ala
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Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly Gly Leu
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Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly Leu Gly
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Trp Thr Phe Asp Arg Ala Glu Ala Gln Lys Leu Ile Glu Ala Leu Gly
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His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg Gly Leu
755 760 765

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Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp Glu His Ala Ala Lys
770 775 780 785

ctc tac gag gac gtc ctc gtc aag gcc aag tac cag tgg tgaacgctag 2579
Leu Tyr Glu Asp Val Leu Val Lys Ala Lys Tyr Gln Trp
790 795

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tattacagag ggcaacgata tgcgccggcg caccggccca actgttgggc cggtcgcaca 2879

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Asp Ala Gly Ile Asp Asp Ala Ala Pro Ala Arg Gln Pro Arg Ala Leu
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Thr Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ser Pro Pro Ala
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Pro Arg Gln Glu Asp Ala Arg Leu Pro Ser Met Asn Gly Met Pro Val
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Val Pro Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro Thr
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Ser Ser Gly Ser Asn Phe Val Pro Ser Ala Ser Ala Pro Gly Ser Asp
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225 230 235 240

Lys Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro Ala
245 250 255

Val Gln Gln Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu Glu
260 265 270

Pro Val Glu Ala Lys Asp Asp Gly Arg Ala Val Ala Asp Asp Ala Gly
275 280 285

Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly Glu
290 295 300

Asn Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp Cys
305 310 315 320

Lys Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala Leu
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Ala Lys Arg Gly His Arg Val Met Val Val Val Pro Arg Tyr Gly Asp
340 345 350

Tyr Glu Glu Ala Tyr Asp Val Gly Val Arg Lys Tyr Tyr Lys Ala Ala

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Asp 385	Phe	Val	Phe	Ile	Asp 390	Ala	Pro	Leu	Phe	Arg 395	His	Arg	Gln	Glu	Asp 400
Ile	Tyr	Gly	Gly	Ser 405	Arg	Gln	Glu	Ile	Met 410	Lys	Arg	Met	Ile	Leu	Phe 415
Cys	Lys	Ala	Ala 420	Val	Glu	Val	Pro	Trp 425	His	Val	Pro	Cys	Gly 430	Gly	Val
Pro	Tyr	Gly 435	Asp	Gly	Asn	Leu	Val 440	Phe	Ile	Ala	Asn	Asp 445	Trp	His	Thr
Ala 450	Leu	Leu	Pro	Val	Tyr	Leu 455	Lys	Ala	Tyr	Tyr	Arg 460	Asp	His	Gly	Leu
Met 465	Gln	Tyr	Thr	Arg	Ser 470	Ile	Met	Val	Ile	His 475	Asn	Ile	Ala	His	Gln 480
Gly	Arg	Gly	Pro	Val 485	Asp	Glu	Phe	Pro	Phe 490	Thr	Glu	Leu	Pro	Glu	His 495
Tyr	Leu	Glu	His 500	Phe	Arg	Leu	Tyr	Asp 505	Pro	Val	Gly	Gly	Glu 510	His	Ala
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Ser 530	Pro	Gly	Tyr	Leu	Trp	Glu 535	Leu	Lys	Thr	Val	Glu 540	Gly	Gly	Trp	Gly
Leu 545	His	Asp	Ile	Ile	Arg 550	Gln	Asn	Asp	Trp	Lys 555	Thr	Arg	Gly	Ile	Val 560
Asn	Gly	Ile	Asp	Asn 565	Met	Glu	Trp	Asn	Pro 570	Glu	Val	Asp	Val	His	Leu 575
Lys	Ser	Asp	Gly 580	Tyr	Thr	Asn	Phe	Ser 585	Leu	Gly	Thr	Leu	Asp 590	Ser	Gly
Lys	Arg	Gln 595	Cys	Lys	Glu	Ala	Leu 600	Gln	Arg	Glu	Leu	Gly 605	Leu	Gln	Val
Arg	Gly 610	Asp	Val	Pro	Leu	Leu 615	Gly	Phe	Ile	Gly	Arg 620	Leu	Asp	Gly	Gln
Lys 625	Gly	Val	Glu	Ile	Ile 630	Ala	Asp	Ala	Met	Pro 635	Trp	Ile	Val	Ser	Gln 640
Asp	Val	Gln	Leu	Val 645	Met	Leu	Gly	Thr	Gly 650	Arg	His	Asp	Leu	Glu	Gly 655
Met	Leu	Arg	His 660	Phe	Glu	Arg	Glu	His 665	His	Asp	Lys	Val	Arg 670	Gly	Trp
Val	Gly	Phe 675	Ser	Val	Arg	Leu	Ala 680	His	Arg	Ile	Thr	Ala 685	Gly	Ala	Asp
Ala 690	Leu	Leu	Met	Pro	Ser	Arg 695	Phe	Glu	Pro	Cys	Gly 700	Leu	Asn	Gln	Leu

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His	Trp	Pro	Pro	Trp	Pro	Pro	Gln	Arg	Thr	Ala	Arg	Asp	Gly	Gly	Val			
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Ala	Ser	Ala	Arg	Gln	Pro	Arg	Ala	Arg	Arg	Gly	Gly	Ala	Ala	Thr	Lys			
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gtc	gcg	gag	cgg	agg	gat	ccc	gtc	aag	acg	ctc	gat	cgc	gac	gcc	gcg	400		
Val	Ala	Glu	Arg	Arg	Asp	Pro	Val	Lys	Thr	Leu	Asp	Arg	Asp	Ala	Ala			
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gaa	ggt	ggc	gcg	ccg	gca	ccg	ccg	gca	ccg	agg	cag	gac	gcc	gcc	cgt	448		
Glu	Gly	Gly	Ala	Pro	Ala	Pro	Pro	Ala	Pro	Arg	Gln	Asp	Ala	Ala	Arg			
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cca	ccg	agt	atg	aac	ggc	acg	ccg	gtg	aac	ggt	gag	aac	aaa	tct	acc	496		
Pro	Pro	Ser	Met	Asn	Gly	Thr	Pro	Val	Asn	Gly	Glu	Asn	Lys	Ser	Thr			

cct Pro	ctc Leu	ttc Phe 395	cga Arg	cac His	cgc Arg	cag Gln	gaa Glu 400	gac Asp	att Ile	tat Tyr	ggg Gly	ggc Gly 405	agc Ser	aga Arg	cag Gln	1312
gaa Glu	att Ile 410	atg Met	aag Lys	cgc Arg	atg Met	att Ile 415	ttg Leu	ttc Phe	tgc Cys	aag Lys	gcc Ala 420	gct Ala	gtc Val	gag Glu	gtt Val	1360
cct Pro 425	tgg Trp	cac His	gtt Val	cca Pro	tgc Cys 430	ggc Gly	ggg Gly	gtc Val	cct Pro	tat Tyr 435	ggg Gly	gat Asp	gga Gly	aat Asn	ctg Leu 440	1408
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tgg Trp	aac Asn 570	ccc Pro	gag Glu	gtg Val	gac Asp	gtc Val	cac His	ctc Leu	aag Lys	tcg Ser	gac Asp 580	ggc Gly	tac Tyr	acc Thr	aac Asn	1840
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gac Asp	gcc Ala	atg Met 635	ccc Pro	tgg Trp	atc Ile	gtg Val	agc Ser 640	cag Gln	gac Asp	gtg Val	cag Gln	ctg Leu 645	gtc Val	atg Met	ctg Leu	2032

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Ser Gly Leu Pro Ala Pro Ala Arg Ala Pro His Pro Ser Thr Gln Asn
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Pro Ser Ser Gly Ser Asn Phe Val Val Ser Ala Ser Ala Pro Arg Leu
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Asp Ile Asp Ser Asp Val Glu Pro Glu Leu Lys Lys Gly Ala Val Ile
225 230 235 240
Val Glu Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro
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Ala Val Gln Glu Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu
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Glu Pro Val Glu Ala Lys Asp Asp Gly Trp Ala Val Ala Asp Asp Ala
275 280 285
Gly Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly
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Glu Asn Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp
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Cys Lys Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala
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Asp	Ile	Tyr	Gly	Gly 405	Ser	Arg	Gln	Glu	Ile 410	Met	Lys	Arg	Met	Ile 415	Leu
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Val	Pro	Tyr 435	Gly	Asp	Gly	Asn	Leu 440	Val	Phe	Ile	Ala	Asn 445	Asp	Trp	His
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Leu 465	Met	Gln	Tyr	Thr	Arg 470	Ser	Ile	Met	Val	Ile 475	His	Asn	Ile	Ala	His 480
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Val	Ser 530	Pro	Gly	Tyr	Leu	Trp 535	Glu	Leu	Lys	Thr	Val 540	Glu	Gly	Gly	Trp
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Gly	Lys 595	Arg	Gln	Cys	Lys	Glu	Ala 600	Leu	Gln	Arg	Glu	Leu 605	Gly	Leu	Gln
Val 610	Arg	Ala	Asp	Val	Pro	Leu 615	Leu	Gly	Phe	Ile	Gly 620	Arg	Leu	Asp	Gly
Gln 625	Lys	Gly	Val	Glu	Ile 630	Ile	Ala	Asp	Ala	Met 635	Pro	Trp	Ile	Val	Ser 640
Gln	Asp	Val	Gln 645	Leu	Val	Met	Leu	Gly	Thr 650	Gly	Arg	His	Asp	Leu 655	Glu
Ser	Met	Leu 660	Arg	His	Phe	Glu	Arg	Glu 665	His	His	Asp	Lys	Val 670	Arg	Gly
Trp	Val 675	Gly	Phe	Ser	Val	Arg	Leu 680	Ala	His	Arg	Ile	Thr 685	Ala	Gly	Ala
Asp 690	Ala	Leu	Leu	Met	Pro	Ser 695	Arg	Phe	Glu	Pro	Cys 700	Gly	Leu	Asn	Gln
Leu 705	Tyr	Ala	Met	Ala	Tyr 710	Gly	Thr	Val	Pro	Val 715	Val	His	Ala	Val	Gly 720
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 Leu Gly His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg
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 Ala Ser Ala Pro Gly Ser Asp Thr Val Ser Asp Val Glu Gln Glu Leu
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 Lys Lys Gly Ala Val Val Val Glu Glu Ala Pro Lys Pro Lys Ala Leu
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 Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp Phe Lys
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 aaa tac att ggt ttc gag gag ccc gtg gag gcc aag gat gat ggc cgg 240
 Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Arg
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 Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp
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 Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala
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 Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala
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 Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val
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Phe Arg His Arg Glu Glu Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile	
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Tyr Tyr Arg Asp His Gly Leu Met Gln Tyr Thr Arg Ser Ile Met Val	
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Ile His Asn Ile Ala His Gln Gly Arg Gly Pro Val Asp Glu Phe Pro	
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Phe Thr Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu Tyr Asp	
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Pro Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met	
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Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys	
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Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp	
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Trp Lys Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu Trp Asn	
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Pro Glu Val Asp Ala His Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser	
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Leu Arg Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala Leu Gln	
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Arg Glu Leu Gly Leu Gln Val Arg Ala Asp Val Pro Leu Leu Gly Phe	
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atg ccc tgg atc gtg agc cag gac gtg cag ctg gtg atg ctg ggc acc 1344
Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val Met Leu Gly Thr
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Gly Arg His Asp Leu Glu Ser Met Leu Gln His Phe Glu Arg Glu His
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His Asp Lys Val Arg Gly Trp Val Gly Phe Ser Val Arg Leu Ala His
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Arg Ile Thr Ala Gly Ala Asp Ala Leu Leu Met Pro Ser Arg Phe Val
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ccg tgc ggg ctg aac cag ctc tac gcc atg gcc tac ggc acc gtc ccc 1536
Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro
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gtc gtg cac gcc gtc ggc ggc ctc agg gac acc gtg ccg ccg ttc gac 1584
Val Val His Ala Val Gly Gly Leu Arg Asp Thr Val Pro Pro Phe Asp
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ccc ttc aac cac tcc ggg ctc ggg tgg acg ttc gac cgc gcc gag gcg 1632
Pro Phe Asn His Ser Gly Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala
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cac aag ctg atc gag gcg ctc ggg cac tgc ctc cgc acc tac cga gac 1680
His Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp
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ttc aag gag agc tgg agg gcc ctc cag gag cgc ggc atg tgc cag gac 1728
Phe Lys Glu Ser Trp Arg Ala Leu Gln Arg Gly Met Ser Gln Asp
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Phe Ser Trp Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu Val Lys
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gcc aag tac cag tgg tgaacgctag ctgctagccg ctccagcccc gcatgcgtgc 1831
Ala Lys Tyr Gln Trp
595

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Lys Lys Gly Ala Val Val Val Glu Glu Ala Pro Lys Pro Lys Ala Leu	35	40	45
Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp Phe Lys	50	55	60
Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Arg	65	70	75
Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp	85	90	95
Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala	100	105	110
Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala	115	120	125
Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val	130	135	140
Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Pro Thr Asp Val Gly Val	145	150	155
Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn Tyr Phe	165	170	175
His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp Ala Pro Leu	180	185	190
Phe Arg His Arg Glu Glu Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile	195	200	205
Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala Val Glu Val Pro Trp	210	215	220
His Val Pro Cys Gly Gly Val Pro Tyr Gly Asp Gly Asn Leu Val Phe	225	230	235
Ile Ala Asn Asp Trp His Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala	245	250	255
Tyr Tyr Arg Asp His Gly Leu Met Gln Tyr Thr Arg Ser Ile Met Val	260	265	270
Ile His Asn Ile Ala His Gln Gly Arg Gly Pro Val Asp Glu Phe Pro	275	280	285
Phe Thr Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu Tyr Asp	290	295	300
Pro Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met	305	310	315
Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys	325	330	335
Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp	340	345	350

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Ser Pro Leu Cys Pro Arg Ser Arg Gln Pro Leu Val Val Val Arg Pro

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Ala	Gly	Arg	Gly	Gly	Leu	Thr	Gln	Pro	Phe	Leu	Met	Asn	Gly	Arg	Phe	
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Thr	Arg	Ser	Arg	Thr	Leu	Arg	Cys	Met	Val	Ala	Ser	Ser	Asp	Pro	Pro	
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aat	agg	aaa	tca	aga	agg	atg	gta	cca	cct	cag	gtt	aaa	gtc	att	tct	244
Asn	Arg	Lys	Ser	Arg	Arg	Met	Val	Pro	Pro	Gln	Val	Lys	Val	Ile	Ser	
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tct	aga	gga	tat	acg	aca	aga	ctc	att	gtt	gaa	cca	agc	aac	gag	aat	292
Ser	Arg	Gly	Tyr	Thr	Thr	Arg	Leu	Ile	Val	Glu	Pro	Ser	Asn	Glu	Asn	
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aca	gaa	cac	aat	aat	cgg	gat	gaa	gaa	act	ctt	gat	aca	tac	aat	gcg	340
Thr	Glu	His	Asn	Asn	Arg	Asp	Glu	Glu	Thr	Leu	Asp	Thr	Tyr	Asn	Ala	
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Leu	Leu	Ser	Thr	Glu	Thr	Ala	Glu	Trp	Thr	Asp	Asn	Arg	Glu	Ala	Glu	
105					110					115					120	
act	gct	aaa	gcg	gac	tcg	tcg	caa	aat	gct	tta	agc	agt	tct	ata	att	436
Thr	Ala	Lys	Ala	Asp	Ser	Ser	Gln	Asn	Ala	Leu	Ser	Ser	Ser	Ile	Ile	
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Gly	Glu	Val	Asp	Val	Ala	Asp	Glu	Asp	Ile	Leu	Ala	Ala	Asp	Leu	Thr	
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gtg	tat	tca	ttg	agc	agt	gta	atg	aag	aag	gaa	gtg	gat	gca	gcg	gac	532
Val	Tyr	Ser	Leu	Ser	Ser	Val	Met	Lys	Lys	Glu	Val	Asp	Ala	Ala	Asp	
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Lys	Ala	Arg	Val	Lys	Glu	Asp	Ala	Phe	Glu	Leu	Asp	Leu	Pro	Ala	Thr	
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aca	ttg	aga	agt	gtg	ata	gta	gat	gtg	atg	gat	cat	aat	ggg	act	gta	628
Thr	Leu	Arg	Ser	Val	Ile	Val	Asp	Val	Met	Asp	His	Asn	Gly	Thr	Val	
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caa	gag	aca	ttg	aga	agt	gtg	ata	gta	gat	gtg	atg	gat	cat	aat	ggg	676
Gln	Glu	Thr	Leu	Arg	Ser	Val	Ile	Val	Asp	Val	Met	Asp	His	Asn	Gly	
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act	gta	caa	gag	aca	ttg	aga	agt	gtg	ata	gta	gat	gtg	atg	gat	gat	724
Thr	Val	Gln	Glu	Thr	Leu	Arg	Ser	Val	Ile	Val	Asp	Val				

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gct Ala	ggg Gly	aat Asn	gat Asp 300	caa Gln	ggc Gly	ata Ile	ttt Phe	aga Arg 305	gca Ala	gat Asp	ttg Leu	tca Ser	gga Gly 310	aat Asn	gtt Val	964
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tct Ser	ata Ile 330	aag Lys	gac Asp	agg Arg	ttt Phe	gag Glu 335	acg Thr	gat Asp	tcg Ser	tca Ser	gga Gly 340	aat Asn	gtt Val	tca Ser	aca Thr	1060
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aca Thr	ttt Phe	gag Glu	gcg Ala	gat Asp 365	ttg Leu	tcg Ser	gga Gly	aat Asn	gct Ala 370	tca Ser	agc Ser	tcg Cys	gca Ala	aca Thr 375	tac Tyr	1156
aga Arg	gaa Glu	gtg Val	gat Asp 380	gat Asp	gtg Val	gtg Val	gat Asp	gaa Glu 385	act Thr	aga Arg	tca Ser	gaa Glu	gag Glu 390	gaa Glu	aca Thr	1204
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cag Gln 425	caa Gln	tat Tyr	cca Pro	gta Val 430	ccg Pro	tct Ser	tca Ser	ttc Phe	tct Ser	atg Met 435	ttg Trp	gac Asp	aag Lys	gct Ala	att Ile 440	1348
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gaa Glu	gaa Glu	caa Gln	ggc Gly 460	aaa Lys	gta Val	aat Asn	ttt Phe	agt Ser 465	gat Asp	aaa Lys	aaa Lys	gac Asp	ctg Leu 470	tca Ser	att Ile	1444
gat Asp	gat Asp	tta Leu 475	cca Pro	gga Gly	caa Gln	aac Asn	caa Gln 480	tcg Ser	atc Ile	att Ile	ggg Gly	tcc Ser 485	tat Tyr	aaa Lys	caa Gln	1492
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tca Ser	att Ile	gtt Val	agt Ser	gtc Val 525	act Thr	gag Glu	caa Gln	aag Lys	cag Gln 530	tcc Ser	ata Ile	gtt Val	gga Gly	ttc Phe 535	cgt Arg	1636

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Ser Gln Asp Leu Ser Ala Val Ser Leu Pro Lys Gln Asn Val Pro Ile	
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Val Gly Thr Ser Arg Glu Gly Gln Thr Lys Gln Val Pro Val Val Asp	
555 560 565	
aga cag gat gca ttg tat gtg aat gga ctg gaa gct aag gag gga gat	1780
Arg Gln Asp Ala Leu Tyr Val Asn Gly Leu Glu Ala Lys Glu Gly Asp	
570 575 580	
cac aca tcc gag aaa act gat gag gat gcg ctt cat gta aag ttt aat	1828
His Thr Ser Glu Lys Thr Asp Glu Asp Ala Leu His Val Lys Phe Asn	
585 590 595 600	
gtt gac aat gtg ttg cgg aag cat cag gca gat aga acc caa gca gtg	1876
Val Asp Asn Val Leu Arg Lys His Gln Ala Asp Arg Thr Gln Ala Val	
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Glu Lys Lys Thr Trp Lys Lys Val Asp Glu Glu His Leu Tyr Met Thr	
620 625 630	
gaa cat cag aaa cgt gct gcc gaa gga cag atg gta gtt aac gag gat	1972
Glu His Gln Lys Arg Ala Ala Glu Gly Gln Met Val Val Asn Glu Asp	
635 640 645	
gag ctt tct ata act gaa att gga atg ggg aga ggt gat aaa att cag	2020
Glu Leu Ser Ile Thr Glu Ile Gly Met Gly Arg Gly Asp Lys Ile Gln	
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cat gtg ctt tct gag gaa gag ctt tca tgg tct gaa gat gaa gtg cag	2068
His Val Leu Ser Glu Glu Glu Leu Ser Trp Ser Glu Asp Glu Val Gln	
665 670 675 680	
tta att gag gat gat gga caa tat gaa gtt gac gag acc tct gtg tcc	2116
Leu Ile Glu Asp Asp Gly Gln Tyr Glu Val Asp Glu Thr Ser Val Ser	
685 690 695	
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Val Asn Val Glu Gln Asp Ile Gln Gly Ser Pro Gln Asp Val Val Asp	
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Pro Gln Ala Leu Lys Val Met Leu Gln Glu Leu Ala Glu Lys Asn Tyr	
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Ser Met Arg Asn Lys Leu Phe Val Phe Pro Glu Val Val Lys Ala Asp	
730 735 740	
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Ser Val Ile Asp Leu Tyr Leu Asn Arg Asp Leu Thr Ala Leu Ala Asn	
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Glu Pro Asp Val Val Ile Lys Gly Ala Phe Asn Gly Trp Lys Trp Arg	
765 770 775	
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Leu Phe Thr Glu Arg Leu His Lys Ser Asp Leu Gly Gly Val Trp Trp	
780 785 790	
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Ser	Cys	Lys 795	Leu	Tyr	Ile	Pro	Lys 800	Glu	Ala	Tyr	Arg	Leu 805	Asp	Phe	Val	
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825					830					835					840	
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	970					975					980					
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985					990					995					1000	
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Ala Gly Thr Thr Val Asp Val Leu Tyr Asn Pro Ser Asn Thr Val Leu			
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Asn Gly Lys Ser Glu Gly Trp Phe Arg Cys Ser Phe Asn Leu Trp Met			
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His Ser Ser Ser Gly Ala Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp			
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Gly Pro Leu Leu Lys Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met			
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Met Asp Phe Val Phe Ser Glu Trp Glu Glu Asp Gly Ile Tyr Asp Asn			
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Arg Asn Gly Met Asp Tyr His Ile Pro Val Ser Asp Ser Ile Glu Thr			
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Cys Leu Asn Gln Ser Ser Val Lys Asp Leu His Leu Tyr Gln Ser Phe			
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Ser Trp Gly Gly Thr Glu Ile Lys Val Trp Val Gly Arg Val Glu Asp			
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Leu Thr Val Tyr Phe Leu Glu Pro Gln Asn Gly Met Phe Gly Val Gly			
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Cys Val Tyr Gly Arg Asn Asp Asp Arg Arg Phe Gly Phe Phe Cys His			
1275	1280	1285	
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Ser Ala Leu Glu Phe Ile Leu Gln Asn Glu Phe Ser Pro His Ile Ile			
1290	1295	1300	
cat tgc cat gat tgg tca agt gct ccg gtc gcc tgg cta tat aag gaa			3988
His Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Tyr Lys Glu			
1305	1310	1315	1320

cac tat tcc caa tcc aga atg gca agc act cgg gtt gta ttt acc atc	4036
His Tyr Ser Gln Ser Arg Met Ala Ser Thr Arg Val Val Phe Thr Ile	
1325 1330 1335	
cac aat ctt gaa ttt gga gca cat tat att ggt aaa gca atg aca tac	4084
His Asn Leu Glu Phe Gly Ala His Tyr Ile Gly Lys Ala Met Thr Tyr	
1340 1345 1350	
tgt gat aaa gcc aca act gtt tct cct aca tat tca agg gac gtg gca	4132
Cys Asp Lys Ala Thr Thr Val Ser Pro Thr Tyr Ser Arg Asp Val Ala	
1355 1360 1365	
ggc cat ggc gcc att gct cct cat cgt gag aaa ttc tac ggc att ctc	4180
Gly His Gly Ala Ile Ala Pro His Arg Glu Lys Phe Tyr Gly Ile Leu	
1370 1375 1380	
aat gga att gat cca gat atc tgg gat ccg tac act gac aat ttt atc	4228
Asn Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile	
1385 1390 1395 1400	
ccg gtc cct tat act tgt gag aat gtt gtc gaa ggc aag aga gct gca	4276
Pro Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala	
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Lys Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro	
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att gtc gga atc atc acc cgt ctg aca gcc cag aag gga atc cac ctc	4372
Ile Val Gly Ile Ile Thr Arg Leu Thr Ala Gln Lys Gly Ile His Leu	
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atc aag cac gca att cac cga act ctc gaa agc aac gga cat gtg gtt	4420
Ile Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly His Val Val	
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Leu Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg	
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Leu Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val	
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cta acc tat gat gag cct ctt tct cac ctg ata tac gct ggc tcg gac	4564
Leu Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ser Asp	
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ttc ata att gtt cct tca atc ttc gaa ccc tgt ggc tta aca caa ctt	4612
Phe Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu	
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gtt gcc atg cgt tat gga tcg atc cct ata gtt cgg aaa act gga gga	4660
Val Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly	
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ctt cac gac aca gtc ttc gac gta gac aat gat aag gac cgg gct cgg	4708
Leu His Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg	
1545 1550 1555 1560	
tct ctt ggt ctt gaa cca aat ggg ttc agt ttc gac gga gcc gac agc	4756
Ser Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser	
1565 1570 1575	

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aat ggc gtg gat tat gcc ctc aac aga gca atc ggc gct tgg ttc gat 4804
Asn Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp
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gcc cgt gat tgg ttc cac tcc ctg tgt aag agg gtc atg gag caa gac 4852
Ala Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp
      1595                      1600                      1605

tgg tcg tgg aac cgg'ccc gca' ctg gac tac att gaa ttg tac cat gcc 4900
Trp Ser Trp Asn Arg Pro Ala Leu Asp Tyr Ile Glu Leu Tyr His Ala
      1610                      1615                      1620

gct cga aaa ttc tgacacccaa ctgaaccaat gacaagaaca agcgcatgtgt 4952
Ala Arg Lys Phe
1625

gggatcgact agtcatacag ggctgtgcag atcgtcttgc ttcagttagt gccctcttca 5012

gttagttcca agcgcactac agtcgtacat agctgaggat cctcttgcct cctaccaggg 5072

ggaacaaagc agaaatgcat gagtgcattg ggaagacttt tatgtatatt gttaaaaaaaa 5132

tttcttttcc ttttcttccc ctgcacctgg aaatgggttaa gcgcatcgcc gagataagaa 5192

ccgcagtgac attctgtgag tagctttgta tattctctca tcttgtgaaa actaatgttc 5252

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<212> PRT
<213> Triticum aestivum

<400> 8
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Gln Pro Leu Val Val Val Arg Pro Ala Gly Arg Gly Gly Leu Thr Gln
      20              25              30

Pro Phe Leu Met Asn Gly Arg Phe Thr Arg Ser Arg Thr Leu Arg Cys
      35              40              45

Met Val Ala Ser Ser Asp Pro Pro Asn Arg Lys Ser Arg Arg Met Val
      50              55              60

Pro Pro Gln Val Lys Val Ile Ser Ser Arg Gly Tyr Thr Thr Arg Leu
      65              70              75              80

Ile Val Glu Pro Ser Asn Glu Asn Thr Glu His Asn Asn Arg Asp Glu
      85              90              95

Glu Thr Leu Asp Thr Tyr Asn Ala Leu Leu Ser Thr Glu Thr Ala Glu
      100             105             110

Trp Thr Asp Asn Arg Glu Ala Glu Thr Ala Lys Ala Asp Ser Ser Gln
      115             120             125

Asn Ala Leu Ser Ser Ser Ile Ile Gly Glu Val Asp Val Ala Asp Glu
      130             135             140

Asp Ile Leu Ala Ala Asp Leu Thr Val Tyr Ser Leu Ser Ser Val Met

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145		150		155		160
Lys Lys Glu Val Asp	Ala Ala Asp	Lys Ala Arg Val	Lys Glu Asp	Ala		
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Phe Glu Leu Asp	Leu Pro Ala Thr	Thr Leu Arg Ser	Val Ile Val Asp			
	180		185		190	
Val Met Asp His Asn	Gly Thr Val	Gln Glu Thr Leu	Arg Ser Val	Ile		
	195		200		205	
Val Asp Val Met Asp	His Asn Gly Thr	Val Gln Glu Thr	Leu Arg Ser			
	210		215		220	
Val Ile Val Asp Val	Met Asp Asp	Ala Ala Asp	Lys Ala Arg	Val Glu		
	225		230		235	
Glu Asp Val Phe Glu	Leu Asp Leu Ser	Gly Asn Ile Ser	Ser Ser Ser	Ala		
	245		250		255	
Thr Thr Val Glu Leu	Asp Ala Val Asp	Glu Val Gly Pro	Val Gln Asp			
	260		265		270	
Lys Phe Glu Ala Thr	Ser Ser Gly Asn	Val Ser Asn Ser	Ala Thr Val			
	275		280		285	
Arg Glu Val Asp Ala	Ser Asp Glu Ala	Gly Asn Asp	Gln Gly Ile Phe			
	290		295		300	
Arg Ala Asp Leu Ser	Gly Asn Val Phe	Ser Ser Ser	Thr Thr Val	Glu		
	305		310		315	
Val Gly Ala Val Asp	Glu Ala Gly Ser	Ile Lys Asp	Arg Phe Glu Thr			
	325		330		335	
Asp Ser Ser Gly Asn	Val Ser Thr Ser	Ala Pro Met Trp	Asp Ala Ile			
	340		345		350	
Asp Glu Thr Val Ala	Asp Gln Asp Thr	Phe Glu Ala Asp	Leu Ser Gly			
	355		360		365	
Asn Ala Ser Ser Cys	Ala Thr Tyr Arg	Glu Val Asp Asp	Val Val Asp			
	370		375		380	
Glu Thr Arg Ser Glu	Glu Glu Thr Phe	Ala Met Asp	Leu Phe Ala Ser			
	385		390		395	
Glu Ser Gly His Glu	Lys His Met Ala	Val Asp Tyr Val	Gly Glu Ala			
	405		410		415	
Thr Asp Glu Glu Glu	Thr Tyr Gln Gln	Gln Tyr Pro Val	Pro Ser Ser			
	420		425		430	
Phe Ser Met Trp Asp	Lys Ala Ile Ala	Lys Thr Gly Val	Ser Leu Asn			
	435		440		445	
Pro Glu Leu Arg Leu	Val Arg Val Glu	Glu Gln Gly Lys	Val Asn Phe			
	450		455		460	
Ser Asp Lys Lys Asp	Leu Ser Ile Asp	Asp Leu Pro Gly	Gln Asn Gln			
	465		470		475	
Ser Ile Ile Gly Ser	Tyr Lys Gln Asp	Lys Ser Ile Ala	Asp Val Ala			
	485		490		495	

Gly	Pro	Thr	Gln	Ser	Ile	Phe	Gly	Ser	Ser	Lys	Gln	His	Arg	Ser	Ile
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Val	Ala	Phe	Pro	Lys	Gln	Asn	Gln	Ser	Ile	Val	Ser	Val	Thr	Glu	Gln
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Lys	Gln	Ser	Ile	Val	Gly	Phe	Arg	Ser	Gln	Asp	Leu	Ser	Ala	Val	Ser
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Leu	Pro	Lys	Gln	Asn	Val	Pro	Ile	Val	Gly	Thr	Ser	Arg	Glu	Gly	Gln
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Thr	Lys	Gln	Val	Pro	Val	Val	Asp	Arg	Gln	Asp	Ala	Leu	Tyr	Val	Asn
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Gly	Leu	Glu	Ala	Lys	Glu	Gly	Asp	His	Thr	Ser	Glu	Lys	Thr	Asp	Glu
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Asp	Ala	Leu	His	Val	Lys	Phe	Asn	Val	Asp	Asn	Val	Leu	Arg	Lys	His
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Gln	Ala	Asp	Arg	Thr	Gln	Ala	Val	Glu	Lys	Lys	Thr	Trp	Lys	Lys	Val
			610				615						620		
Asp	Glu	Glu	His	Leu	Tyr	Met	Thr	Glu	His	Gln	Lys	Arg	Ala	Ala	Glu
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Gly	Gln	Met	Val	Val	Asn	Glu	Asp	Glu	Leu	Ser	Ile	Thr	Glu	Ile	Gly
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Met	Gly	Arg	Gly	Asp	Lys	Ile	Gln	His	Val	Leu	Ser	Glu	Glu	Glu	Leu
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Ser	Trp	Ser	Glu	Asp	Glu	Val	Gln	Leu	Ile	Glu	Asp	Asp	Gly	Gln	Tyr
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Glu	Val	Asp	Glu	Thr	Ser	Val	Ser	Val	Asn	Val	Glu	Gln	Asp	Ile	Gln
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Gly	Ser	Pro	Gln	Asp	Val	Val	Asp	Pro	Gln	Ala	Leu	Lys	Val	Met	Leu
			705				710						715		
Gln	Glu	Leu	Ala	Glu	Lys	Asn	Tyr	Ser	Met	Arg	Asn	Lys	Leu	Phe	Val
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Phe	Pro	Glu	Val	Val	Lys	Ala	Asp	Ser	Val	Ile	Asp	Leu	Tyr	Leu	Asn
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Arg	Asp	Leu	Thr	Ala	Leu	Ala	Asn	Glu	Pro	Asp	Val	Val	Ile	Lys	Gly
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Ala	Phe	Asn	Gly	Trp	Lys	Trp	Arg	Leu	Phe	Thr	Glu	Arg	Leu	His	Lys
			770				775						780		
Ser	Asp	Leu	Gly	Gly	Val	Trp	Trp	Ser	Cys	Lys	Leu	Tyr	Ile	Pro	Lys
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Glu	Ala	Tyr	Arg	Leu	Asp	Phe	Val	Phe	Phe	Asn	Gly	Arg	Thr	Val	Tyr
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Glu	Asn	Asn	Gly	Asn	Asn	Asp	Phe	Cys	Ile	Gly	Ile	Glu	Gly	Thr	Met
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Asn	Glu	Asp	Leu	Phe	Glu	Asp	Phe	Leu	Val	Lys	Glu	Lys	Gln	Arg	Glu
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Leu Glu Lys Leu Ala Met Glu Glu Ala Glu Arg Arg Thr Gln Thr Glu
 850 855 860
 Glu Gln Arg Arg Arg Lys Glu Ala Arg Ala Ala Asp Glu Ala Val Arg
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 Ala Gln Ala Lys Ala Glu Ile Glu Ile Lys Lys Lys Lys Leu Gln Ser
 885 890 895
 Met Leu Ser Leu Ala Arg Thr Cys Val Asp Asn Leu Trp Tyr Ile Glu
 900 905 910
 Ala Ser Thr Asp Thr Arg Gly Asp Thr Ile Arg Leu Tyr Tyr Asn Arg
 915 920 925
 Asn Ser Arg Pro Leu Ala His Ser Thr Glu Ile Trp Met His Gly Gly
 930 935 940
 Tyr Asn Asn Trp Thr Asp Gly Leu Ser Ile Val Glu Ser Phe Val Lys
 945 950 955 960
 Cys Asn Asp Lys Asp Gly Asp Trp Trp Tyr Ala Asp Val Ile Pro Pro
 965 970 975
 Glu Lys Ala Leu Val Leu Asp Trp Val Phe Ala Asp Gly Pro Ala Gly
 980 985 990
 Asn Ala Arg Asn Tyr Asp Asn Asn Ala Arg Gln Asp Phe His Ala Ile
 995 1000 1005
 Leu Pro Asn Asn Asn Val Thr Glu Glu Gly Phe Trp Ala Gln Glu Glu
 1010 1015 1020
 Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu Arg Arg Glu Lys Glu Glu
 025 1030 1035 1040
 Thr Met Lys Arg Lys Ala Glu Arg Ser Ala Asn Ile Lys Ala Glu Met
 1045 1050 1055
 Lys Ala Lys Thr Met Arg Arg Phe Leu Leu Ser Gln Lys His Ile Val
 1060 1065 1070
 Tyr Thr Glu Pro Leu Glu Ile Arg Ala Gly Thr Thr Val Asp Val Leu
 1075 1080 1085
 Tyr Asn Pro Ser Asn Thr Val Leu Asn Gly Lys Ser Glu Gly Trp Phe
 1090 1095 1100
 Arg Cys Ser Phe Asn Leu Trp Met His Ser Ser Gly Ala Leu Pro Pro
 105 1110 1115 1120
 Gln Lys Met Val Lys Ser Gly Asp Gly Pro Leu Leu Lys Ala Thr Val
 1125 1130 1135
 Asp Val Pro Pro Asp Ala Tyr Met Met Asp Phe Val Phe Ser Glu Trp
 1140 1145 1150
 Glu Glu Asp Gly Ile Tyr Asp Asn Arg Asn Gly Met Asp Tyr His Ile
 1155 1160 1165
 Pro Val Ser Asp Ser Ile Glu Thr Glu Asn Tyr Met Arg Ile Ile His
 1170 1175 1180
 Ile Ala Val Glu Met Ala Pro Val Ala Lys Val Gly Gly Leu Gly Asp

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185	1190	1195	1200
Val Val Thr Ser Leu Ser Arg Ala Ile Gln Asp Leu Gly His Thr Val	1205	1210	1215
Glu Val Ile Leu Pro Lys Tyr Asp Cys Leu Asn Gln Ser Ser Val Lys	1220	1225	1230
Asp Leu His Leu Tyr Gln Ser Phe Ser Trp Gly Gly Thr Glu Ile Lys	1235	1240	1245
Val Trp Val Gly Arg Val Glu Asp Leu Thr Val Tyr Phe Leu Glu Pro	1250	1255	1260
Gln Asn Gly Met Phe Gly Val Gly Cys Val Tyr Gly Arg Asn Asp Asp	1265	1270	1275
Arg Arg Phe Gly Phe Phe Cys His Ser Ala Leu Glu Phe Ile Leu Gln	1285	1290	1295
Asn Glu Phe Ser Pro His Ile Ile His Cys His Asp Trp Ser Ser Ala	1300	1305	1310
Pro Val Ala Trp Leu Tyr Lys Glu His Tyr Ser Gln Ser Arg Met Ala	1315	1320	1325
Ser Thr Arg Val Val Phe Thr Ile His Asn Leu Glu Phe Gly Ala His	1330	1335	1340
Tyr Ile Gly Lys Ala Met Thr Tyr Cys Asp Lys Ala Thr Thr Val Ser	1345	1350	1355
Pro Thr Tyr Ser Arg Asp Val Ala Gly His Gly Ala Ile Ala Pro His	1365	1370	1375
Arg Glu Lys Phe Tyr Gly Ile Leu Asn Gly Ile Asp Pro Asp Ile Trp	1380	1385	1390
Asp Pro Tyr Thr Asp Asn Phe Ile Pro Val Pro Tyr Thr Cys Glu Asn	1395	1400	1405
Val Val Glu Gly Lys Arg Ala Ala Lys Arg Ala Leu Gln Gln Lys Phe	1410	1415	1420
Gly Leu Gln Gln Thr Asp Val Pro Ile Val Gly Ile Ile Thr Arg Leu	1425	1430	1435
Thr Ala Gln Lys Gly Ile His Leu Ile Lys His Ala Ile His Arg Thr	1445	1450	1455
Leu Glu Ser Asn Gly His Val Val Leu Leu Gly Ser Ala Pro Asp His	1460	1465	1470
Arg Ile Gln Gly Asp Phe Cys Arg Leu Ala Asp Ala Leu His Gly Val	1475	1480	1485
Tyr His Gly Arg Val Lys Leu Val Leu Thr Tyr Asp Glu Pro Leu Ser	1490	1495	1500
His Leu Ile Tyr Ala Gly Ser Asp Phe Ile Ile Val Pro Ser Ile Phe	1505	1510	1515
Glu Pro Cys Gly Leu Thr Gln Leu Val Ala Met Arg Tyr Gly Ser Ile	1525	1530	1535

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gat gtc gtc atc aaa gga gca ttc aat ggt tgg aaa tgg agg ctt ttc Asp Val Val Ile Lys Gly Ala Phe Asn Gly Trp Lys Trp Arg Leu Phe 195 200 205	624
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gga ata gaa ggc act atg aat gaa gat ctg ttt gag gat ttc ttg gtt Gly Ile Glu Gly Thr Met Asn Glu Asp Leu Phe Glu Asp Phe Leu Val 260 265 270	816
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Gln	Asp	Phe	His	Ala	Ile	Leu	Pro	Asn	Asn	Asn	Val	Thr	Glu	Glu	
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Phe	Trp	Ala	Gln	Glu	Glu	Gln	Asn	Ile	Tyr	Thr	Arg	Leu	Leu	Gln	
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Arg	Arg	Glu	Lys	Glu	Glu	Thr	Met	Lys	Arg	Lys	Ala	Glu	Arg	Ser	
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Asn	Ile	Lys	Ala	Glu	Met	Lys	Ala	Lys	Thr	Met	Arg	Arg	Phe	Leu	
485				490				495							
tcc	cag	aaa	cac	att	gtt	tat	acc	cga	acc	gnc	ttg	aaa	tac	gtg	1536
Ser	Gln	Lys	His	Ile	Val	Tyr	Thr	Arg	Thr	Xaa	Leu	Lys	Tyr	Val	
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gga	acc	aca	gtg	gat	gtg	cta	tac	aat	ccc	tct	aac	aca	gtg	cta	1584
Gly	Thr	Thr	Val	Asp	Val	Leu	Tyr	Asn	Pro	Ser	Asn	Thr	Val	Leu	
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Pro	Leu	Leu	Lys	Ala	Thr	Val	Asp	Val	Pro	Pro	Asp	Ala	Tyr	Met	
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Asn	Gly	Met	Asp	Tyr	His	Ile	Pro	Val	Ser	Asp	Ser	Ile	Glu	Thr	
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Asn	Tyr	Met	Arg	Ile	Ile	His	Ile	Ala	Val	Glu	Met	Ala	Pro	Val	
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ttg	aac														

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Cys	His	Asp	Trp	Ser	Ser	Ala	Pro	Val	Ala	Trp	Leu	Tyr	Lys	Glu	His									
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Tyr	Ser	Gln	Ser	Arg	Met	Ala	Ser	Thr	Arg	Val	Val	Phe	Thr	Ile	His									
755								760								765								
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Asn	Leu	Glu	Phe	Gly	Ala	His	Tyr	Ile	Gly	Lys	Ala	Met	Thr	Tyr	Cys									
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gat	aaa	gcc	aca	act	gtt	tct	cct	aca	tat	tca	agg	gac	gtg	gca	ggc	2400								
Asp	Lys	Ala	Thr	Thr	Val	Ser	Pro	Thr	Tyr	Ser	Arg	Asp	Val	Ala	Gly									
790								795								800								
cat	ggc	gcc	att	gct	cct	cat	cgt	gag	aaa	ttc	tac	ggc	att	ctc	aat	2448								
His	Gly	Ala	Ile	Ala	Pro	His	Arg	Glu	Lys	Phe	Tyr	Gly	Ile	Leu	Asn									
805								810								815								
gga	att	gat	cca	gat	atc	tgg	gat	ccg	tac	act	gac	aat	ttt	atc	ccg	2496								
Gly	Ile	Asp	Pro	Asp	Ile	Trp	Asp	Pro	Tyr	Thr	Asp	Asn	Phe	Ile	Pro									
820								825								830								
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Val	Pro	Tyr	Thr	Cys	Glu	Asn	Val	Val	Glu	Gly	Lys	Arg	Ala	Ala	Lys									
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agg	gcc	ttg	cag	cag	aag	ttt	gga	tta	cag	caa	act	gat	gtc	cct	att	2592								
Arg	Ala	Leu	Gln	Gln	Lys	Phe	Gly	Leu	Gln	Gln	Thr	Asp	Val	Pro	Ile									
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gtc	gga	atc	atc	acc	cgt	ctg	aca	gca	cag	aag	gga	atc	cac	ctc	atc	2640								
Val	Gly	Ile	Ile	Thr	Arg	Leu	Thr	Ala	Gln	Lys	Gly	Ile	His	Leu	Ile									
865								870								875								
aag	cac	gca	att	cac	cga	acc	ctc	gag	agc	aat	gga	caa	gtg	gtt	ttg	2688								
Lys	His	Ala	Ile	His	Arg	Thr	Leu	Glu	Ser	Asn	Gly	Gln	Val	Val	Leu									
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Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu Val	
945 950 955 960	
gcc atg cgt tat gga tgc atc cct ata gtt cgg aaa acc gga gga ctt	2928
Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly Leu	
965 970 975	
tac gac act gtc ttc gac gta gac aat gat aag gac cgg gct cgg tct	2976
Tyr Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg Ser	
980 985 990	
ctt ggt ctt gaa cca aat ggg ttc agt ttc gac gga gcc gac agc aat	3024
Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser Asn	
995 1000 1005	
ggc gtg gat tat gcc ctg aac aga gca atc ggc gct tgg ttc gat gcc	3072
Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp Ala	
1010 1015 1020	
cgt gat tgg ttc cac tcc ctg tgt aag agg gtc atg gag caa gac tgg	3120
Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp Trp	
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Ser Trp Asn Arg Pro Ala Leu Asp Tyr Ile Glu Leu Tyr His Ala Ala	
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Arg Lys Phe	
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<212> PRT
<213> Triticum aestivum
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Ser Glu Lys Thr Asp Glu Asp Ala Leu His Val Lys Phe Asn Val Asp
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Asn Val Leu Arg Lys His Gln Ala Asp Arg Thr Gln Ala Val Glu Lys
      35          40          45

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Lys	Thr	Trp	Lys	Lys	Val	Asp	Glu	Glu	His	Leu	Tyr	Met	Thr	Glu	His
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Ser	Ile	Thr	Glu	Ile	Gly	Met	Gly	Arg	Gly	Asp	Lys	Ile	Gln	His	Val
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			100					105					110		
Glu	Asp	Asp	Gly	Gln	Tyr	Glu	Val	Asp	Glu	Thr	Ser	Val	Ser	Val	Asn
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Val	Glu	Gln	Asp	Ile	Gln	Gly	Ser	Pro	Gln	Asp	Val	Val	Asp	Pro	Gln
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Ala	Leu	Lys	Val	Met	Leu	Gln	Glu	Leu	Ala	Glu	Lys	Asn	Tyr	Ser	Met
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Arg	Asn	Lys	Leu	Phe	Val	Phe	Pro	Glu	Val	Val	Lys	Ala	Asp	Ser	Val
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Ile	Asp	Leu	Tyr	Leu	Asn	Arg	Asp	Leu	Thr	Ala	Leu	Ala	Asn	Glu	Pro
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		195					200					205			
Thr	Glu	Arg	Leu	His	Lys	Ser	Asp	Leu	Gly	Gly	Val	Trp	Trp	Ser	Cys
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Lys	Leu	Tyr	Ile	Pro	Lys	Glu	Ala	Tyr	Arg	Leu	Asp	Phe	Val	Phe	Phe
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Lys	Glu	Lys	Gln	Arg	Glu	Leu	Glu	Lys	Leu	Ala	Met	Glu	Glu	Ala	Glu
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Arg	Arg	Thr	Gln	Thr	Glu	Glu	Gln	Arg	Arg	Arg	Lys	Glu	Ala	Arg	Ala
	290					295					300				
Ala	Asp	Glu	Ala	Val	Arg	Ala	Gln	Ala	Lys	Ala	Glu	Ile	Glu	Ile	Lys
305					310					315					320
Lys	Lys	Lys	Leu	Gln	Ser	Met	Leu	Ser	Leu	Ala	Arg	Thr	Cys	Val	Asp
				325					330					335	
Asn	Leu	Trp	Tyr	Ile	Glu	Ala	Ser	Thr	Asp	Thr	Arg	Gly	Asp	Thr	Ile
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Arg	Leu	Tyr	Tyr	Asn	Arg	Asn	Ser	Arg	Pro	Leu	Ala	His	Ser	Thr	Glu
		355					360					365			
Ile	Trp	Met	His	Gly	Gly	Tyr	Asn	Asn	Trp	Ser	Asp	Gly	Leu	Ser	Ile
	370					375					380				
Val	Glu	Ser	Phe	Val	Lys	Cys	Asn	Asp	Lys	Asp	Gly	Asp	Trp	Trp	Tyr

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385		390		395		400
Ala Asp Val Ile	Pro Pro Glu Lys Ala	Leu Val Leu Asp Trp Val Phe				
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Ala Asp Gly Pro	Ala Gly Asn Ala Arg Asn Tyr Asp Asn Asn Ala Arg					
	420	425			430	
Gln Asp Phe His	Ala Ile Leu Pro Asn Asn Asn Val Thr Glu Glu Gly					
	435	440			445	
Phe Trp Ala Gln	Glu Glu Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu					
	450	455			460	
Arg Arg Glu Lys	Glu Glu Thr Met Lys Arg Lys Ala Glu Arg Ser Ala					
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Asn Ile Lys Ala	Glu Met Lys Ala Lys Thr Met Arg Arg Phe Leu Leu					
	485	490			495	
Ser Gln Lys His	Ile Val Tyr Thr Arg Thr Xaa Leu Lys Tyr Val Pro					
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Gly Thr Thr Val	Asp Val Leu Tyr Asn Pro Ser Asn Thr Val Leu Asn					
	515	520			525	
Gly Lys Ser Glu	Gly Trp Phe Arg Cys Ser Phe Asn Leu Trp Met His					
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Ser Ser Gly Ala	Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp Gly					
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Pro Leu Leu Lys	Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met Met					
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Asp Phe Val Phe	Ser Glu Trp Glu Glu Asp Gly Ile Tyr Asp Asn Arg					
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Asn Gly Met Asp	Tyr His Ile Pro Val Ser Asp Ser Ile Glu Thr Glu					
	595	600			605	
Asn Tyr Met Arg	Ile Ile His Ile Ala Val Glu Met Ala Pro Val Ala					
	610	615			620	
Lys Val Gly Gly	Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala Ile					
	625	630			635	640
Gln Asp Leu Gly	His Thr Val Glu Val Ile Leu Pro Lys Tyr Asp Cys					
	645	650			655	
Leu Asn Gln Ser	Ser Val Lys Asp Leu His Leu Tyr Gln Ser Phe Ser					
	660	665			670	
Trp Gly Gly Thr	Glu Ile Lys Val Trp Val Gly Arg Val Glu Asp Leu					
	675	680			685	
Thr Val Tyr Phe	Leu Glu Pro Gln Asn Gly Met Phe Gly Val Gly Cys					
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Val Tyr Gly Arg	Asn Asp Asp Arg Arg Phe Gly Phe Phe Cys His Ser					
	705	710			715	720
Ala Leu Glu Phe	Ile Leu Gln Asn Glu Phe Ser Pro His Ile Ile His					
	725	730			735	

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Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Tyr Lys Glu His
 740 745 750
 Tyr Ser Gln Ser Arg Met Ala Ser Thr Arg Val Val Phe Thr Ile His
 755 760 765
 Asn Leu Glu Phe Gly Ala His Tyr Ile Gly Lys Ala Met Thr Tyr Cys
 770 775 780
 Asp Lys Ala Thr Thr Val Ser Pro Thr Tyr Ser Arg Asp Val Ala Gly
 785 790 795 800
 His Gly Ala Ile Ala Pro His Arg Glu Lys Phe Tyr Gly Ile Leu Asn
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 Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile Pro
 820 825 830
 Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala Lys
 835 840 845
 Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro Ile
 850 855 860
 Val Gly Ile Ile Thr Arg Leu Thr Ala Gln Lys Gly Ile His Leu Ile
 865 870 875 880
 Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly Gln Val Val Leu
 885 890 895
 Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg Leu
 900 905 910
 Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val Leu
 915 920 925
 Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ser Asp Phe
 930 935 940
 Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu Val
 945 950 955 960
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 Tyr Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg Ser
 980 985 990
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 995 1000 1005
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 Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp Trp
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<211> 728

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<212> DNA

<213> Triticum sp.

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<211> 2446

<212> DNA

<213> Triticum sp.

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<212> DNA
<213> Triticum sp.

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<211> 892
<212> DNA
<213> Triticum sp.

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ccccgttgca aaggtaatat aattctaagg ctagtcttctt tgatgcgagg cg 892

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<211> 871
<212> DNA
<213> Triticum sp.

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<212> DNA
<213> Triticum sp.

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